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A Diverse Range of Bacterial and Eukaryotic Chitinases Hydrolyzes the LacNAc (Gal β 1–4GlcNAc) and LacdiNAc (GalNAc β 1–4GlcNAc) Motifs Found on Vertebrate and Insect Cells^{*[5]}

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Rikki F. Frederiksen^{†1}, Yayoi Yoshimura^{§1}, Birgit G. Storgaard^{†§1}, Dafni K. Paspaliari[‡], Bent O. Petersen^{§2}, Kowa Chen[¶], Tanja Larsen[‡], Jens Ø. Duus^{§3}, Hanne Ingmer[‡], Nicolai V. Bovin^{||}, Ulrika Westerlind^{**}, Ola Blixt^{††}, Monica M. Palcic[§], and Jørgen J. Leisner^{†4}

From the [†]Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Grønnegaardsvej 10, 1870 Frederiksberg C., Denmark, [§]Carlsberg Laboratory, Gamle Carlsberg Vej 10, 1799 Copenhagen V, Denmark, [¶]Copenhagen Center for Glycomics, Department of Cellular and Molecular Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3, 2200 Kbh. N., Denmark, ^{||}Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, Moscow 117997, Russian Federation, ^{**}Gesellschaft zur Förderung der Analytischen Wissenschaften e.V., ISAS-Leibnitz Institute for Analytical Sciences, Otto-Hahn-Strasse 6b, D-44227 Dortmund, Germany, and ^{††}Department of Chemistry, University of Copenhagen, 6:4:T422, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark

Background: The biological role(s) of chitinases other than hydrolysis/metabolism of chitin is currently not well understood.

Results: A *Salmonella* chitinase binds *N*-acetylactosamine (LacNAc), a common component of mammalian glycoconjugates. Furthermore, five bacterial and eukaryotic chitinases hydrolyze terminal LacNAc and LacdiNAc from model substrates.

Conclusion: LacdiNAc and LacNAc- glycans are substrates for chitinases.

Significance: Vertebrate and invertebrate molecules carrying LacNAc and LacdiNAc motifs are potential chitinase targets.

There is emerging evidence that chitinases have additional functions beyond degrading environmental chitin, such as involvement in innate and acquired immune responses, tissue remodeling, fibrosis, and serving as virulence factors of bacterial pathogens. We have recently shown that both the human chitotriosidase and a chitinase from *Salmonella enterica* serovar Typhimurium hydrolyze LacNAc from Gal β 1–4GlcNAc β -tetramethylrhodamine (LacNAc-TMR (Gal β 1–4GlcNAc β (CH₂)₈-CONH(CH₂)₂NHCO-TMR)), a fluorescently labeled model substrate for glycans found in mammals. In this study we have examined the binding affinities of the *Salmonella* chitinase by carbohydrate microarray screening and found that it binds to a range of compounds, including five that contain LacNAc structures. We have further examined the hydrolytic specificity of this enzyme and chitinases from *Sodalis glossinidius* and *Polysphondylium pallidum*, which are phylogenetically related to the *Salmonella* chitinase, as well as unrelated chitinases from *Listeria monocytogenes* using the fluorescently labeled substrate analogs LacdiNAc-TMR (GalNAc β 1–4GlcNAc β -TMR), LacNAc-

TMR, and LacNAc β 1–6LacNAc β -TMR. We found that all chitinases examined hydrolyzed LacdiNAc from the TMR aglycone to various degrees, whereas they were less active toward LacNAc-TMR conjugates. LacdiNAc is found in the mammalian glycome and is a common motif in invertebrate glycans. This substrate specificity was evident for chitinases of different phylogenetic origins. Three of the chitinases also hydrolyzed the β 1–6 bond in LacNAc β 1–6LacNAc β -TMR, an activity that is of potential importance in relation to mammalian glycans. The enzymatic affinities for these mammalian-like structures suggest additional functional roles of chitinases beyond chitin hydrolysis.

Chitinases are ubiquitous enzymes found in archaea, bacteria, fungi, plants, and animals. They hydrolyze chitin, the second most abundant polysaccharide in nature. Chitin is a linear polymer comprised of β 1–4-linked *N*-acetyl-D-glucosamine (GlcNAc) units and serves as a structural polymer in crustacean shells, in the cell walls of fungi, and in the exoskeletons of arthropods. The biological roles of some chitinases are well established, such as in chitin cycling in the marine environment by *Vibrio cholerae* and other *Vibrio* spp. (1), in yeast cell division (2), and in cuticle turnover and nutrient digestion in arthropods (3). Many plant chitinases are induced upon microbial infection, and some have the potential to inhibit the growth of fungal pathogens, hence displaying a defensive role (4, 5). However, the biological functions of numerous chitinases are

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[5] This article contains supplemental Table 1 and Fig. 1.

[†] These authors contributed equally to this work.

² Current address: Novo Nordisk, A/S Novo Nordisk Park, DK-2760 Måløv, Denmark.

³ Current address: Dept. of Chemistry, Technical University of Denmark, Kemitorvet, Bldg. 201, DK-2800 Kgs. Lyngby, Denmark.

⁴ To whom correspondence should be addressed. Tel.: 45-3533-2768; Fax: 45-3533-2755; E-mail: jji@sund.ku.dk.

TABLE 1

Overview of chitinases used in this study

Protein	Gene	Species	Source
StChiA	STM0018 ^a	<i>Salmonella</i> Typhimurium LT2	This study
StChiAΔCBM	SL0018ΔCBM ^a (1936→2097)	<i>Salmonella</i> Typhimurium SL1344	(14)
StChiA E223Q	STM0018, G697C	<i>Salmonella</i> Typhimurium LT2	This study
StChiA E223QΔCBM	STM0018, G697C, ΔCBM (1936→2097)	<i>Salmonella</i> Typhimurium LT2	This study
LmChiA	lmo1883	<i>L. monocytogenes</i> EGD-e	(21)
LmChiB	lmo0105	<i>L. monocytogenes</i> EGD-e	This study
LmChiBΔCBM	lmo0105 ΔCBM (2130→2265)	<i>L. monocytogenes</i> EGD-e	This study
PpChitinase	EFA83839	<i>P. pallidum</i> PN500	This study
SgChiA	SG1474	<i>S. glossinidius</i> str. morsitans	This study
<i>S. griseus</i> chitinase	Unspecified	<i>S. griseus</i>	Sigma
<i>T. viride</i> chitinase ^b	Unspecified	<i>T. viride</i>	Sigma

^a The STM0018 and SL0018 genes have identical nucleotide sequence.^b According to manufacturer, a mixture of two chitinases.

still unknown, especially in organisms that rarely come into contact with chitin.

One eukaryotic example is chitinases encoded by cellular slime molds, such as *Dictyostelium* spp. and *Polysphondylium* spp. These organisms utilize mainly bacteria as a food source, and consumption of chitin-containing yeast supports growth only poorly. There are also examples of plant chitinases that have defensive physiological roles that are attributed to ribosome inactivating activity (6, 7). Humans produce two active chitinases, chitotriosidase and acidic mammalian chitinase (AMCase), that can be linked to a variety of disorders including lysosomal storage disease, asthma, and neurological diseases (8–11). Finally, it is now emerging that for several bacterial pathogens and symbionts, chitinases may be important as virulence or symbiotic factors as much as, or rather than, being important for chitin metabolism. Examples include enzymes produced by *Legionella pneumophila*, *Listeria monocytogenes*, and possibly *Salmonella* Typhimurium (12–14). Understanding the exact role of chitinases in infectious processes is, however, limited by the fact that their target substrates in the host remain unidentified.

The gene expression of *Salmonella* Typhimurium chitinase, StChiA, is up-regulated during infection of macrophage and epithelial cells (15, 16). We recently found that the catalytic domain of this enzyme (StChiAΔCBM) cleaves *N*-acetylglucosamine (LacNAc;⁵ (Galβ1–4GlcNAc)) from the tetramethylrhodamine (TMR, see Fig. 1) fluorescently labeled substrate analog LacNAc-TMR in addition to hydrolyzing chitin, chitooligosaccharides, and the chitin pseudo-substrate *p*NP-(GlcNAc)₂ (14). Hydrolysis occurs with retention of configuration and is consistent with the TMR-linker occupying the + subsites of the enzyme with the Gal and GlcNAc of LacNAc in the –2 and –1 subsites, respectively. This is analogous to the hydrolysis of *p*NP-(GlcNAc)₂, where *p*-nitrophenyl (*p*NP) occupies the +1 subsite and (GlcNAc)₂ is bound to the –2 and –1 subsites. Because *N*-acetylglucosamine is a common component of mammalian glycans, it constitutes a possible chitinase

target. We have also shown that human chitotriosidase cleaves LacNAc and LacdiNAc (GalNAcβ1–4GlcNAc) from LacNAc-TMR and LacdiNAc-TMR, respectively, with the latter having a turnover comparable to that of *p*NP-chitotriosidase (17).

In this study we have cloned and characterized chitinases, which are phylogenetically related to the *Salmonella* enzyme StChiA, from the bacterial insect endosymbiont *Sodalis glossinidius* and the eukaryotic slime mold *Polysphondylium pallidum* (see Table 1 and Fig. 2). These three enzymes share the common characteristic that they lack a signal peptide but are still predicted to be secretory proteins (14).

For comparison we have included the phylogenetically unrelated chitinases LmChiA and LmChiB (18) from *L. monocytogenes* (see Table 1 and Fig. 2) and the commercially available chitinases from the actinobacterium *Streptomyces griseus* and the mold *Trichoderma viride*. The chitinases included in the present study all belong to the CAZy family 18 glycoside hydrolases, although it should be noted that the two commercial available chitinases from *T. viride* and *S. griseus* remain uncharacterized in this regard.

We observed that the *Salmonella* chitinase StChiA was able to bind to potential glycan targets in hosts by use of a carbohydrate microarray. In addition, we found that all enzymes surveyed (with one exception) hydrolyzed LacdiNAc from fluorescently labeled conjugates, whereas several of the enzymes hydrolyzed LacNAc from analogous conjugates that are substrate analogs of glycans found in mammals.

EXPERIMENTAL PROCEDURES

Phylogenetic Analysis—The amino acid sequences of *Salmonella* Typhimurium SL1344 StChiA (SL0018), *S. glossinidius* str. morsitans SgChiA (SG1474), *P. pallidum* PpChitinase (EFA83839), *L. monocytogenes* LmChiA (lmo1883), and LmChiB (lmo0105) (Table 1) were from the GenBankTM database of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/). Protein domain predictions including determination of the glycosyl hydrolase family 18 (GH_18) domains were obtained from the SMART website. Signal peptide predictions were performed using SignalP 4.0 from the Centre for Biological Sequence Analysis, Biocentrum, Technical University of Denmark. GH_18 domains of sequences representative of bacterial chitinases in addition to representatives of eukaryotic chitinases were included in the phylogenetic anal-

⁵ The abbreviations used are: LacNAc, *N*-acetylglucosamine; Galβ1–4GlcNAc; LacNAc-TMR, Galβ1–4GlcNAcβ(CH₂)₈CONH(CH₂)₂NHCO-TMR; TMR, tetramethylrhodamine; LacdiNAc-TMR, GalNAcβ1–4GlcNAcβ(CH₂)₈CONH(CH₂)₂NHCO-TMR; Type I-TMR, Galβ1–3GlcNAcβ(CH₂)₈CONH(CH₂)₂NHCO-TMR; *p*NP-(GlcNAc)₂, Galβ1–4GlcNAcβ-*p*NP; LacNAcβ1–6LacNAcβ-TMR, Galβ1–4GlcNAcβ1-6 Galβ1–4GlcNAcβ(CH₂)₂NHCO-TMR; St, *Salmonella* Typhimurium; Pp, *P. pallidum*; Lm, *L. monocytogenes*; Sg, *S. glossinidius*; *p*NP, *p*-nitrophenyl.

ysis. The phylogenetic tree (see Fig. 2) based on the corresponding GH_18 domains was constructed by the neighbor-joining method using ClustalX Version 1.81 (19) and the Mega version 4 (20) software packages with default settings.

Production and Purification of Chitinases—The nucleotide sequences of the chitinase genes SG1474 of *S. glossinidius* str. morsitans and EFA83839 of *P. pallidum* PN500 were codon-optimized for expression in *Escherichia coli*, and the genes were synthesized with an N-terminal His₆ tag flanked by NcoI/XhoI restriction sites (Epoch Biolabs, Missouri City, TX). The synthetic genes were cloned into pET15b expression vectors, which were transformed into chemically competent *E. coli* TOP10 cells (Invitrogen) for long term storage. For protein expression the vector carrying SG1474 was transformed into *E. coli* BL21(DE3) cells (Novagen, Darmstadt, Germany), whereas the vector carrying EFA83839 was transformed into *E. coli* BL21/TUNERTM(DE3) cells (Novagen). 10 ng of pET-15b-SG1474 or EFA83839 were added to 50 μ l of *E. coli* TOP10, *E. coli* BL21(DE3), or *E. coli* BL21/TunerTM(DE3) cells. The reaction mixture was incubated on ice for 30 min, heat-shocked at 42 °C for 30 s, and incubated on ice for an additional 2 min, then 200 μ l of Luria broth (LB; Merck) was added. The reaction mixture was incubated at 37 °C for 60 min with shaking and plated on LB agar plates containing 100 μ g/ml carbenicillin and incubated overnight at 37 °C. Transformants were re-streaked on carbenicillin-containing LB plates. The following day colonies were grown in LB and stored as glycerol stocks.

The *L. monocytogenes* EGDe *chiB* gene (*lmo0105*) and a truncated version of *chiB* (*lmo1883 Δ CBM*) lacking the C-terminal carbohydrate binding domain (amino acids 711–755) were amplified through PCR with primers designed to exclude the respective signal peptide sequences but including a His₆ tag at the N terminus. The PCR products were cloned into the pET-46 Ek/LIC vector and introduced into *E. coli* BL21(DE3) according to the manufacturer's instructions (Novagen pET-46 E/LIC vector kit). The absence of mis- and nonsense mutations was confirmed by sequencing (Macrogen, Seoul, Korea). The *E. coli* BL21(DE3) strain used for expression of the *L. monocytogenes* chitinase, *LmChiA* (*lmo1883*), was from a previous study (21). An *E. coli* BL21(DE3) strain carrying a *Salmonella* chitinase gene without the chitin-binding domain (*SL0018 Δ CBM*) and used for expression of *StChiA Δ CBM* was prepared in a previous study (14). Expression vectors for the production of *Salmonella* wild type chitinase, *StChiA*, and the inactive enzyme variants *StChiA* E223Q and *StChiA* E223Q Δ CBM were purchased from Epoch life sciences Inc. (Missouri City, TX). In brief, *StChiA* E223Q was obtained by substituting the active site glutamate with a glutamine residue by site-directed mutagenesis (E223Q, GAA→CAA), whereas *ChiA* E223Q Δ CBM was obtained by a further truncation from amino acid 646. The synthetic genes were cloned into NdeI/XhoI restriction sites of the pETb15 expression vector carrying a His-tag coding sequence. The resulting vectors was transformed into *E. coli* BL21(DE3) as previously described.

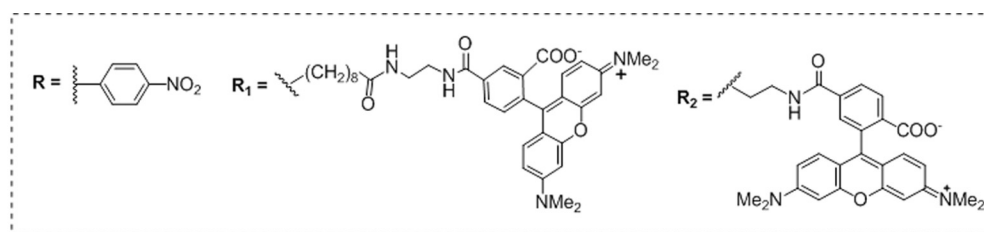
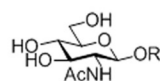
For protein purification, up to 1 liter of *E. coli* BL21(DE3) cells were grown at 30 °C to an *A*₆₀₀ of 0.4 before induction with 1 mM isopropyl 1-thio- β -D-galactopyranoside. After induction, growth was continued for 21 h at 30 °C. For improved recovery and

purification of the *Polysphondylium* chitinase, *PpChitinase*, *E. coli* BL21/TunerTM(DE3) cells were grown at 37 °C to an *A*₆₀₀ of 0.6 and placed on ice to cool before induction with 0.3 mM isopropyl 1-thio- β -D-galactopyranoside. After induction, growth was continued for 21 h at 22 °C. The *E. coli* BL21(DE3) and/or *E. coli* BL21/Tuner cells were harvested by centrifugation and resuspended in 10 volumes of loading buffer, 20 mM MOPS, pH 7.2, containing 0.5 M NaCl and 5 mM imidazole. The cells were disrupted using a Constant Systems cell disruptor at 4 °C with a pressure of 1.36 kilobars. The lysate was centrifuged at 4 °C for 1.5 h at 48,000 \times g, and the filtered supernatant was applied to a 1- or 5-ml HisTrap HP column (GE Healthcare) with a flow rate of 1 or 5 ml/min, respectively. For the *Listeria* enzymes, 1–2-ml columns of nickel-nitrilotriacetic acid-agarose (Qia-gen) were used with a flow rate of 1 ml/min. All columns were washed with 100 ml of loading buffer before being eluted with 30 ml of 100 mM MOPS, pH 7.8, 0.5 M NaCl, and 0.5 M imidazole. For the *Sodalis* chitinase *SgChiA* and the *Salmonella* *StChiA Δ CBM*, fractions containing enzyme were combined, the buffer was exchanged to loading buffer by dialysis, and the enzymes were rechromatographed on a 1-ml HisTrap column with an imidazole gradient from 5 to 250 mM imidazole to obtain ultrapure enzymes. For all enzymes, the fractions containing enzyme were dialyzed against 50 mM sodium phosphate buffer, pH 6.0, at 4 °C and concentrated in a Vivaspin (10,000 Da cutoff for *LmChiA* and 30,000-Da cutoff for other enzymes). The protein concentration of the sample was determined by the Bradford method using a commercial kit (Bio-Rad) with bovine γ -globulin as a protein reference standard. Alternatively, protein concentration was measured with Qubit 2.0 fluorometer (Invitrogen) according to the manufacturer's protocol. Commercially available enzymes of *S. griseus* (Sigma) and *T. viride* (a mixture of two chitinases; Sigma) were dissolved according to manufacturer's instructions and included in the study as reference enzymes.

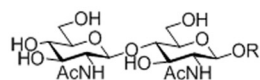
For the enzymes from *Salmonella*, *Sodalis*, *Polysphondylium*, and *Listeria*, purified in this study, 12–73 mg of recombinant protein were obtained per liter of cell culture after chromatography on HisTrap columns. The purity of proteins after isolation assessed by SDS-PAGE was as found previously (14).

Glycan Array Scanning—Glycan arrays were fabricated as described previously (22, 23) and contained 317 carbohydrate ligands (supplemental Table 1). The chips were first treated for 30 min with blocking buffer (50 mM ethanolamine in 50 mM borate buffer, pH 8.0) and subsequently for 30 min with 0.1 M phosphate-buffered saline (PBS) (10 mM Na₂HPO₄, 10 mM NaH₂PO₄, 138 mM NaCl, and 2.7 mM KCl, pH 7.4) (Sigma) containing 0.1% Tween 20 (ICN, MP Biomedicals, Santa Ana, CA). 1 ml of 200 μ g/ml enzyme (*StChiA*, *StChiA* E223Q, and *StChiA* E223Q Δ CBM) diluted in PLI-P buffer (0.5 M NaCl, 3 mM KCl, 1.5 mM KH₂PO₄, 6.5 mM Na₂HPO₄, 1% BSA, 1% Triton-X-100, pH 7.4), pH 7.4, was added to the slide and incubated for 60 min at 37 °C. The slide was washed in 1 \times PBS (0.01 M Na₂HPO₄, 0.01 M NaH₂PO₄, 0.138 M NaCl, and 0.0027 M KCl, pH 7.4) (Sigma) containing 0.1% Tween 20 (ICN), then 1 ml of rabbit anti-*ChiA* polyclonal antibody (CovalAb UK Ltd, Cambridge, UK) diluted to 1/1000 in PLI-P buffer was added, and

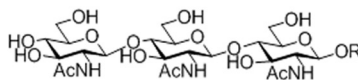
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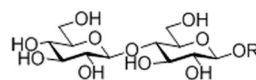
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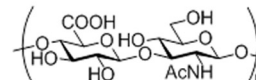
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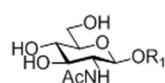
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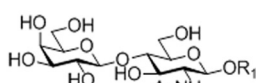
Hyaluronic acid



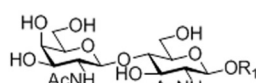
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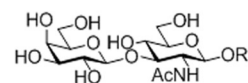
LacNAc-TMR



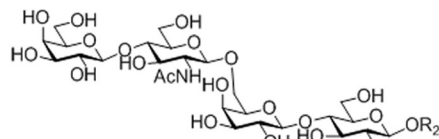
LacdiNAc-TMR



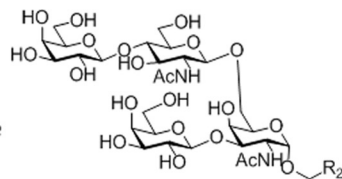
Type I-TMR



LacNAcβ1,6-LacNAc-TMR



Extended Core2-TMR



LacNAc-Man-TMR

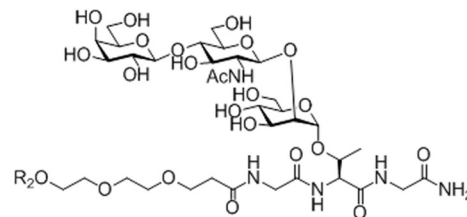


FIGURE 1. Overview of substrates used in this study.

the slide was incubated for 60 min at 22 °C. The slide was washed with 1×PBS, then 1 ml of labeled secondary antibody (Cy5 goat anti-rabbit IgG (H+L); Invitrogen) diluted 1/500 in PLI-P buffer was added before incubation for 60 min at 22 °C. The slide was then washed with 1×PBS, spin-dried, and stored in the dark until analysis. Fluorescence intensities were detected using a ScanArray 5000 (PerkinElmer Life Sciences) confocal scanner, and image analyses were carried out by using ScanArray Express 3.0 and the fixed 70-μm-diameter rings method as well as Microsoft EXCEL software.

Examination of Enzyme Activity—A survey of the activity of chitinases was initially carried out with the pNP chitin pseudo-substrates: pNP-GlcNAc, pNP-(GlcNAc)₂, pNP-(GlcNAc)₃, and the cellulose pseudo-substrate 4-nitrophenyl-β-D-cellobioside whose structures are shown in Fig. 1. In the survey, 5 μl of enzyme (40–2000 μg/ml) diluted in 50 mM sodium phosphate buffer, pH 6.0, and 45 μl of 1.8 mM substrate dissolved in 50 mM sodium phosphate buffer, pH 6.0, were incubated at 30 °C. To limit the conversion of substrates to a maximum of 15–20%, the following enzyme concentrations were used: SgChiA (400 μg/ml), StChiAΔCBM (200 μg/ml), PpChitinase (100 μg/ml), LmChiA (40 μg/ml), LmChiB (200 μg/ml),

LmChiBΔCBM (200 μg/ml), T. viride mixture (67 μg/ml) and S. griseus (400 μg/ml). 50-μl samples were removed after 30 min, and the reaction was quenched by adding 250 μl of 0.4 M sodium carbonate (Sigma S2127). Absorbance at 405 nm was measured in a plate reader and corrected for absorption using a control sample with added sodium phosphate buffer, pH 6.0, instead of enzyme. After the initial screening, pNP-(GlcNAc)₂ absorption values were converted into concentrations by the use of a p-nitrophenol (Sigma) standard curve. Conversion rates were normalized against protein concentration and are listed as pmol/min/mg of protein.

Kinetic parameters for pNP-(GlcNAc)₂ were determined for SgChiA (23 μg/ml) and PpChitinase (20 μg/ml) by measuring initial rates of reaction at eight different substrate concentrations ranging from 0.007 to 1.8 mM during 25–120 min of incubation at 30 °C. *K_m* and *V_{max}* were calculated using the software GraphPad Prism 4.0 (GraphPad Software), and *k_{cat}* was estimated by dividing *V_{max}* by enzyme concentration.

The activity of chitinases toward hyaluronic acid (Sigma, H5388) was tested in a standard enzymatic assay of hyaluronidase (Sigma) using hyaluronidase (bovine testes, Sigma H3506)

as a positive control. Samples were prepared according to the manufacturer's instructions and incubated at 37 °C for up to 40 h, and absorbance was read as % transmittance (%T) at 600 nm.

Fluorescently labeled model substrates had a TMR tag covalently linked to carbohydrates via a hydrophobic linker ((carbohydrate) β -O-(CH₂)₈CONH(CH₂)₂NHCO-TMR) (24, 25). The structures are shown in Fig. 1. Initial screening was carried out by TLC analysis where 1 μ l of GlcNAc-TMR (1 mM), LacNAc-TMR (20 mM), LacdiNAc-TMR (20 mM), or Type I-TMR (Gal β 1-3GlcNAc-TMR, 1 mM) were incubated with 5 μ l of enzyme at ambient temperature for 2–24 h. Reaction progress was monitored by removing 1- μ l aliquots for thin-layer chromatography on silica gel plates developed with CHCl₃/MeOH/H₂O (65/35/5) as described previously (17). The TMR-labeled compounds, which are brightly colored red/purple, can be visualized by eye. The *T. viride* chitinase mixture was used as a positive control for monitoring GlcNAc-TMR and LacdiNAc-TMR hydrolysis.

After screening by TLC, conversion rates of LacNAc-TMR and LacdiNAc-TMR for all chitinases were determined by monitoring product formation with 5 mM substrate at 30 °C by capillary electrophoresis. The reaction volume was 4 μ l in 50 mM sodium phosphate buffer, pH 6.0, and enzyme concentrations were 0.6–2.5 mg/ml. Aliquots (1 μ l) were removed at 120 min and quenched with 50 μ l of CE running buffer (50 mM borate, pH 9.3, and 150 mM sodium dodecyl sulfate). Analyses were performed on an automated PrinCE 560 CE system from PrinCE Technologies B.V. (Emmen, The Netherlands). Conversion rates were normalized against protein concentration and are listed as pmol/min/mg of protein. The enzyme concentrations for assay with LacdiNAc/LacNAc were: SgChiA (2000 μ g/ml), StChiA Δ CBM (67/400 μ g/ml), PpChitinase (2000 μ g/ml), LmChiA (2000 μ g/ml), LmChiB (200/2000 μ g/ml), LmChiB Δ CBM (200/2000 μ g/ml), *T. viride* mixture (20/200 μ g/ml), *S. griseus* (1000 μ g/ml). An independent study of conversion rates of LacNAc-TMR, LacdiNAc-TMR, and pNP-(GlcNAc)₂ for all chitinases was performed using the same substrate concentrations as above but with fixed enzyme concentrations of 200 μ g/ml.

Kinetic parameters for LacNAc-TMR and LacdiNAc-TMR were determined for *Sodalis*, *Salmonella*, and *Polysphondylium* enzymes by monitoring product formation with 8 different concentrations of substrate ranging from 0.080 to 10.5 mM at 30 °C by capillary electrophoresis. The reaction volume was 10 μ l in 50 mM sodium phosphate buffer, pH 6.0, and enzyme concentrations were 0.6–2.5 mg/ml. Aliquots (1 μ l) were removed at 12–120 min and quenched with 50 μ l of CE running buffer as described above. Analyses were performed as described above. Separations were carried out in an uncoated fused-silica capillary of 75- μ m inner diameter with CE running buffer. TMR-labeled compounds were detected and quantitated using an Argos 250B fluorescence detector (Flux Instruments, Basel, Switzerland) equipped with an excitation filter of 546.1/10 nm and an emission filter of 570 nm. All experiments were carried out at a normal polarity, i.e. inlet anodic. Data were processed with the PrinCE 7.0 software.

Ultra-HPLC-mass spectrometry analysis was employed to screen extended substrates terminating in LacNAc including LacNAc β 1-6LacNAc β -TMR, LacNAc β 1-6(Gal β 1-3)GalNAc α -TMR (extended core 2), and LacNAc β 1-2-Man-TMR substrates (Fig. 1), which are only available in limited amounts (26, 27). Assays were carried out in 4 μ l containing 50 mM sodium phosphate buffer, pH 6.0, 1 μ g of each enzyme, and 5 pmol of TMR-substrate. After overnight incubation at room temperature, a 1- μ l aliquot was removed, mixed with 300 μ l of buffer (10 mM ammonium formate, pH 4.5 (22%), acetonitrile (88%) (v/v)) for analysis by ultra-HPLC-MS as previously described (17).

Nuclear Magnetic Resonance—Substrates, either pNP-(GlcNAc)₂, (GlcNAc)₃, LacNAc-TMR, or LacdiNAc-TMR (0.4–1 mg), were dissolved in 0.7 ml of buffer, transferred to 5-ml nuclear magnetic resonance (NMR) tubes, and standard one-dimensional ¹H NMR spectra of substrates were acquired before and after the addition of 5–50 μ l of undiluted SgChiA and StChiA Δ CBM (LacdiNAc-TMR only). NMR spectra were recorded at 15 or 25 °C on a Bruker Avance 800 instrument and analyzed as previously described (14). Deuterated buffer was prepared by lyophilizing 5 ml of 50 mM sodium phosphate buffer, pH 6.0, followed by the addition of 2 ml of ²H₂O to the dried solids, re-lyophilization, and suspension in 5 ml of ²H₂O. Spectra were recorded at 799.3 MHz using tetramethylsilane (δ = 0 ppm) as the internal standard with a 32 scan composite presaturation.

RESULTS

Phylogenetic Analyses—A phylogenetic analysis of selected family 18 glycosyl hydrolase catalytic domains revealed that they cluster roughly into three distinct groups (colored in red, green, and blue, respectively, in Fig. 2). The groups colored in green and blue fit into Cluster A according to the analysis of Karlsson and Stenlid (28). Catalytic domains in these two groups in general carry a chitin-insertion domain conferring a deep substrate binding cleft, which suggests exo-activity (29). The group colored in blue contains StChiA, SgChiA, and PpChitinase from *Salmonella*, *Sodalis*, and *Polysphondylium*, respectively, and is characterized by a lack of signal peptides, yet all are predicted to be secretory proteins. On the other hand, the green group containing LmChiB from *Listeria* and also human chitinases in general carry signal peptides. The group colored in red (Fig. 2) containing LmChiA fit into group IV in cluster B according to Karlsson and Stenlid (28). The red group is characterized by the lack of a chitin-insertion domain, which confers a shallow substrate binding cleft suggesting endo-activity (29).

Glycan Array Scanning—The glycan array scanning was performed to screen for ligand binding by the full-length active *Salmonella* StChiA enzyme as well as inactive variants with or without the chitin binding domain. Average background signal was below 1000 relative fluorescence units (RFU), and average signals above 3000 RFU were considered as positives. The glycan array scanning showed binding of the inactive *Salmonella* chitinases (StChiA E223Q and StChiA E223Q Δ CBM) to GlcNAc-containing glycans including the chitin oligosaccha-

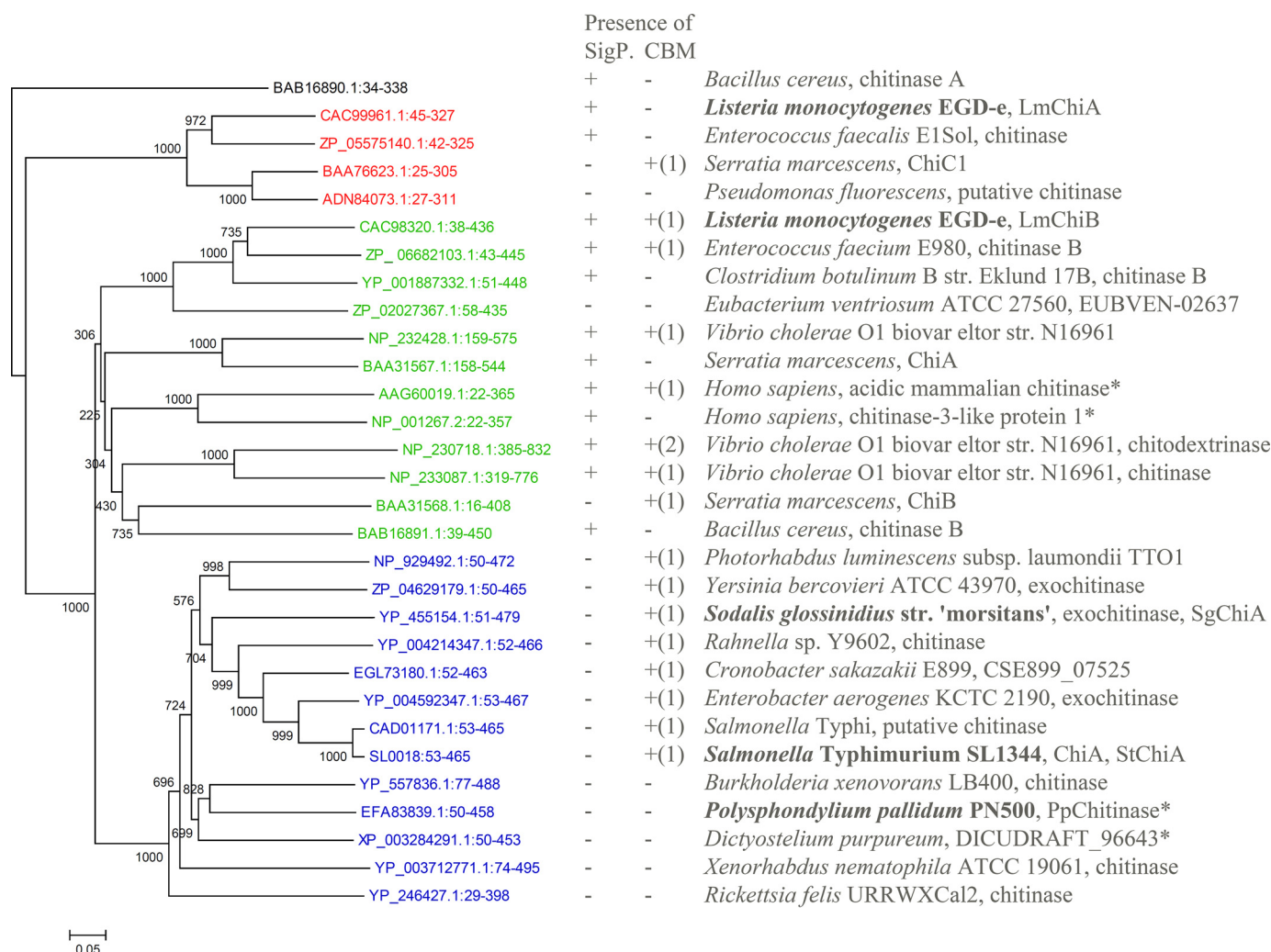


FIGURE 2. Phylogenetic tree of GH_18 domains including *Salmonella* Typhimurium ChiA, *L. monocytogenes* ChiA and ChiB, *S. glossinidius* ChiA, and *P. pallidum* chitinase (all in bold) with representative sequences for similar domains in other species and strains. Also shown is the presence of N-terminal signal peptide (SigP) and chitin binding modules (CBM). Bar, 0.05 substitutions per nucleotide position. Tree labels are colored to indicate phylogenetic grouping. Descriptions of enzymes are from the sequence annotations. Eukaryotic sequences (*Homo sapiens*, *P. pallidum*, and *Dictyostelium purpureum*) are marked with an asterisk (*).

TABLE 2

Affinity of *Salmonella* StChiA and the inactive variants StChiA E223Q and StChiA E223QΔCBM for glycans immobilized on a glass slide (glycan array screening)

—, average signal is below 3000 relative fluorescence units (RFU); +, average signal is >3000 RFU; ++, average signal is >10,000 RFU; +++, average signal is >15,000 RFU. TF, Thomsen-Friedenreich Galβ1–3GalNAc; LN, N-acetylglucosamine (LacNAc); sp2, -O(CH₂)₂NH₂; sp3, -O(CH₂)₃NH₂; sp4, -NHCOCH₂NH₂.

Glycan no.	Glycan name	StChiA	StChiA E223Q	StChiA E223Q ΔCBM
493	Chitopentaose (GlcNAcβ1–4) ₅ β-sp4	—	+++	+++
503	Chitohexaose (GlcNAcβ1–4) ₆ β-sp4	—	+++	+++
384	LNβ3'LN-Galβ1–4GlcNAcβ1–3Galβ1–4GlcNAcβ-sp2	+++	+++	++
385	LNβ3'LN-Galβ1–4GlcNAcβ1–3Galβ1–4GlcNAcβ-sp3	+++	++	+++
420	(4'SuLN)β3'LN 4-O-SuGalβ1–4GlcNAcβ1–3Galβ1–4GlcNAcβ-sp3	++	+	+
498	(LN) ₃ (Galβ1–4GlcNAcβ1–3) ₃ -sp3	+++	+	+
229	Ab3'LN Galβ1–3Galβ1–4GlcNAcβ-sp4	+	—	—
88	TF _β -antigen Galβ1–3GalNAcβ-sp3	+	—	—
89	TF-antigen Galβ1–3GalNAcα-sp3	+	—	—
110	Maltose Glcα1–4Glcβ-sp3	+	—	—
390	Maltotetraose (Glcα1–4) ₄ β-sp4	++	—	—

rides chitopentaose and chitohexaose (glycan no. 493 and 503; Table 2, supplemental Table 1). The active enzyme (StChiA) did not bind to the latter two compounds, unlike the inactive variants, showing that hydrolytic activity interfered with glycan binding. The active enzyme also bound to a range of non-chi-

tinous substrates with binding to five LacNAc-containing compounds (compounds No. 384, 385, 420, 498, 229; Table 2, supplemental Table 1) as well as TF (Thomsen-Friedenreich antigen Galβ1–3GalNAc) antigen and TF_β antigen structures (glycan number 88 and 89, respectively; Table 2 and supple-

TABLE 3

Overview of the initial substrate activity screening of *Salmonella*, *Sodalis*, *Polysphondylium* and *Listeria* chitinases monitored by spectrophotometric pNP-assay or TLC

For the pNP assay: +, positive reaction ($ABS_{405} > 0.1$); (+), weak positive reaction ($ABS_{405} = 0.07-0.1$); -, negative reaction ($ABS_{405} < 0.06$). For the TLC assay: +, clear red/purple band visible to the eye; (+), weak red/purple band but visible to the eye; -, no band visible to the eye. For the hyaluronidase activity assay: %T, % transmittance at 600 nm.

Assay	Substrate ^a	StChiAΔCBM	SgChiA	PpChitinase	LmChiA	LmChiB	LmChiBΔCBM	<i>T. viride</i> mixture	<i>S. griseus</i> chitinase
pNP assay	pNP-GlcNAc	-	-	-	- ^b	NT	NT	+	-
	pNP-(GlcNAc) ₂	+	+	+	+	+	+	+	+
	pNP-(GlcNAc) ₃	+	(+)	NT	+ ^b	NT	NT	+	(+)
	pNP-cellobioside	-	-	-	- ^b	NT	NT	-	-
TLC assay	GlcNAc-TMR	-	-	-	-	-	NT	+	-
	LacNAc-TMR	+	+	-	-	(+)	(+)	-	-
	LacdiNAc-TMR	+	+	+	+	+	+	+	-
	Type I-TMR	-	-	-	-	-	NT	-	-
%T	Hyaluronic acid	-	-	-	-	-	-	(+)	-

^a pNP-GlcNAc: 4-nitrophenyl-*N*-acetyl-β-D-glucosaminide; pNP-(GlcNAc)₂, 4-nitrophenyl-*N,N'*-diacetyl-β-D-chitobioside; pNP-(GlcNAc)₃, 4-nitrophenyl-β-D-*N,N',N''*-triacylchitotriose; pNP-cellobioside, 4-nitrophenyl-β-D-cellobioside; NT, not tested.

^b Not tested in this study. Data are from Leisner *et al.* (21).

mental Table 1). The inactive enzymes only bound to some of these substrates (compound numbers 384, 385, 420, and 498) suggesting that changes in the active site also affected binding.

Substrate Specificity and Kinetic Parameters—The activity spectrum of chitinases was initially screened by the use of the chitin pseudo-substrates pNP-GlcNAc, pNP-(GlcNAc)₂, pNP-(GlcNAc)₃, and pNP-cellobioside, the mammalian and insect model substrates LacNAc-TMR and LacdiNAc-TMR, Type I-TMR (Galβ1-3GlcNAc-TMR isomer of LacNAc-TMR), and hyaluronic acid (Table 3). All of the tested chitinases demonstrated activity toward the chitin analogs pNP-(GlcNAc)₂ and pNP-(GlcNAc)₃. None of the chitinases tested was active against pNP-cellobioside, and only the reference enzyme *T. viride* chitinase mixture demonstrated activity toward pNP-GlcNAc and hyaluronic acid. All chitinases except the *S. griseus* chitinase were to varying degrees active toward the substrate LacdiNAc-TMR. The StChiAΔCBM and SgChiA and to some extent also the LmChiB chitinase were active against LacNAc-TMR. The latter results were confirmed by use of capillary electrophoresis except for LmChiB (Tables 4 and 5). None of the chitinases were active against the isomeric Type I-TMR (Table 3).

Conversion rates in pmol/min/mg of protein were determined for all chitinases toward pNP-(GlcNAc)₂, LacNAc-TMR, and LacdiNAc-TMR, and clear differences among the chitinases were observed. For pNP-(GlcNAc)₂, the LmChiA was the fastest converter (after the reference *T. viride* chitinase) followed by StChiAΔCBM. The *Salmonella* enzyme StChiAΔCBM was the fastest converter of LacdiNAc-TMR (Table 4).

We further studied the kinetics of the three phylogenetically related enzymes. Both StChiAΔCBM and SgChiA hydrolyzed LacNAc-TMR and LacdiNAc-TMR with greater activity toward LacdiNAc-TMR for both enzymes as seen by the higher k_{cat} value (Table 5, Fig. 3). StChiAΔCBM had a k_{cat} for LacdiNAc-TMR of 0.18 s^{-1} , which is 25% that of the k_{cat} for the chitinase substrate pNP-(GlcNAc)₂, whereas k_{cat}/K_m was 20% that of pNP-(GlcNAc)₂. The k_{cat} for LacNAc-TMR hydrolysis was 0.046 s^{-1} about 7% of pNP-(GlcNAc)₂. For SgChiA, the k_{cat} value for LacdiNAc-TMR was 0.035 s^{-1} compared with a k_{cat} value of 0.015 s^{-1} for LacNAc-TMR hydrolysis. The PpChitinase also showed some activity against LacdiNAc-TMR,

TABLE 4

Conversion rates for hydrolysis of LacdiNAc-TMR, LacNAc-TMR, and pNP-(GlcNAc)₂ by *Salmonella*, *Sodalis*, *Polysphondylium*, and *Listeria* chitinases

Enzyme	Conversion rates of substrate		
	LacdiNAc-TMR ^a	LacNAc-TMR ^a	pNP-(GlcNAc) ₂ ^b
	pmol/min/mg enzyme		
SgChiA	8.3 ± 0.0	5.1 ± 2.6	320 ± 60
StChiAΔCBM	151 ± 3	28.0 ± 0.1	2460 ± 40
PpChitinase	5.0 ± 0.3	0.0 ± 0.0	1570 ± 350
LmChiA	5.0 ± 0.1	0.6 ± 0.6	5660 ± 520
LmChiB	33 ± 13	0.1 ± 0.2	1750 ± 50
LmChiBΔCBM	47.1 ± 0.0	0.0 ± 0.1	1630 ± 80
<i>T. viride</i> chitinase mixture	1232 ± 50	0.2 ± 0.3	6700 ± 80
<i>S. griseus</i> chitinase	0.9 ± 0.7	0.3 ± 0.3	1380 ± 40

^a 5 mM substrate, 30 °C, 120 min, analysis by capillary electrophoresis.

^b 1.8 mM substrate, 30 °C, 30 min analysis by spectrophotometry.

although it was slower than the StChiAΔCBM and SgChiA as seen from the low k_{cat} value of 0.007 s^{-1} . On the other hand, the PpChitinase was more active against pNP-(GlcNAc)₂ than StChiAΔCBM and SgChiA as seen from the high k_{cat} value of 10.3 s^{-1} compared with 0.7 and 3.1 s^{-1} for StChiAΔCBM and SgChiA, respectively (Table 5).

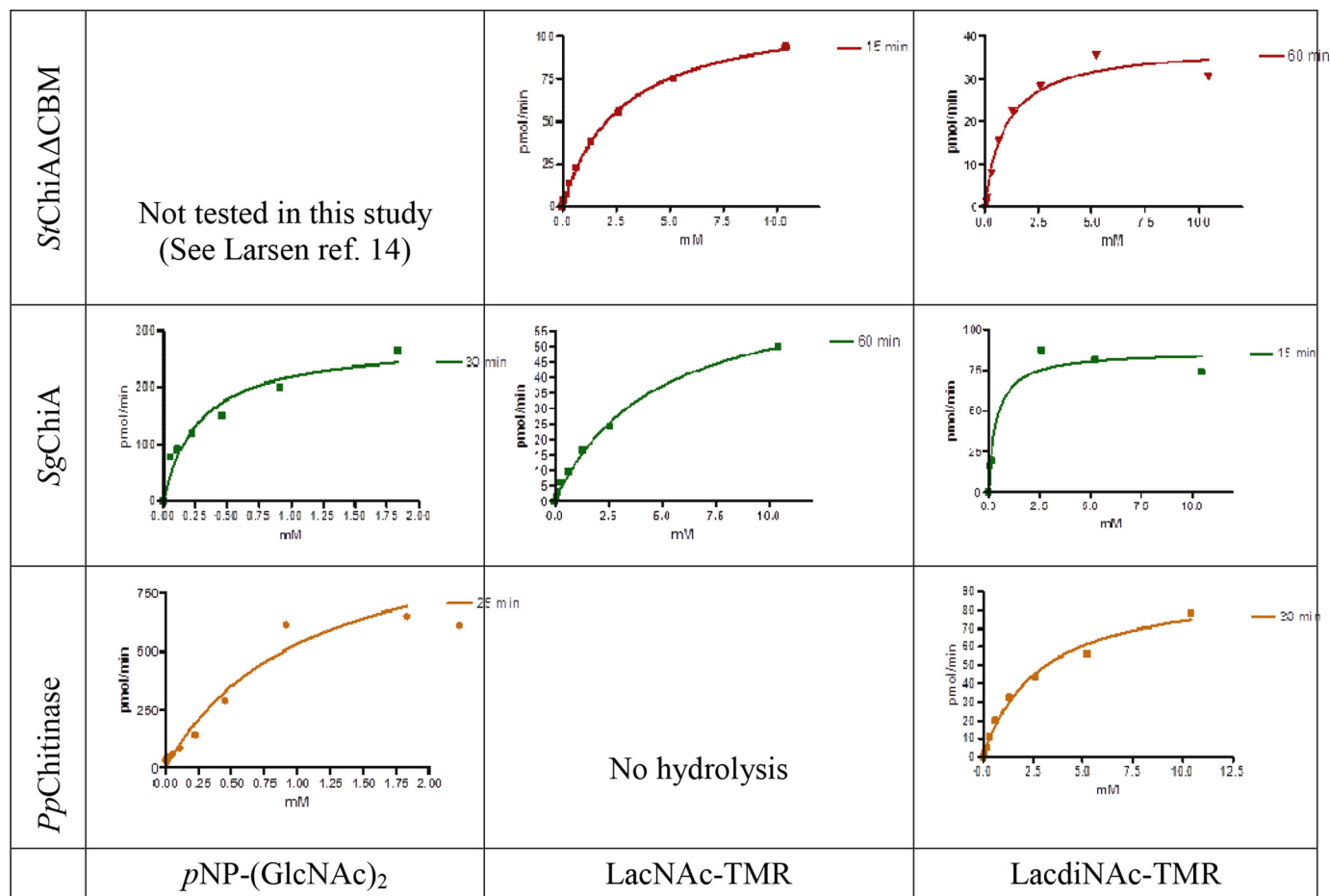
Hydrolysis of larger substrates with terminal LacNAc (Fig. 1) was monitored by ultra-HPLC-MS due to the limited amounts of material available. Only LacNAcβ1-6LacNAcβ-TMR was a substrate. It was hydrolyzed by stepwise removal of terminal LacNAc forming LacNAc-TMR disaccharide. Subsequent hydrolysis of LacNAc-TMR can yield the TMR linker (Fig. 4B). The LacNAc disaccharides that are cleaved are not detected by the fluorescence detector used in our analysis. The *Sodalis* SgChiA enzyme was the most efficient with respect to hydrolysis with essentially complete conversion to a mixture of LacNAc-TMR (65%) and the TMR-linker (33%). LmChiB was the least efficient enzyme with conversion to 3% each of LacNAc-TMR and the TMR-linker. LmChiA showed no activity with this extended substrate, whereas the StChiAΔCBM enzyme only removed the terminal LacNAc giving 62% LacNAc-TMR (Fig. 4, A and C).

Nuclear Magnetic Resonance Studies of *Sodalis* and *Salmonella* Chitinases with pNP-(GlcNAc)₂, pNP-(GlcNAc)₃, LacNAc-TMR, and LacdiNAc-TMR—LacdiNAc-TMR and LacNAc-TMR were shown to be substrates of SgChiA and StChiAΔCBM with release of LacdiNAc and LacNAc, respectively, as moni-

TABLE 5

Kinetic constants for *Salmonella*, *Sodalis*, and *Polysphondylium* chitinases with pNP-(GlcNAc)₂, LacNAc-TMR, and LacdiNAc-TMR

Enzyme	pNP-(GlcNAc) ₂			LacNAc-TMR ^a			LacdiNAc-TMR ^z		
	<i>K_m</i>	<i>k_{cat}</i>	<i>k_{cat}/K_m</i>	<i>K_m</i>	<i>k_{cat}</i>	<i>k_{cat}/K_m</i>	<i>K_m</i>	<i>k_{cat}</i>	<i>k_{cat}/K_m</i>
	<i>mM</i>	<i>s</i> ⁻¹	<i>mM</i> ⁻¹ <i>s</i> ⁻¹	<i>mM</i>	<i>s</i> ⁻¹	<i>mM</i> ⁻¹ <i>s</i> ⁻¹	<i>mM</i>	<i>s</i> ⁻¹	<i>mM</i> ⁻¹ <i>s</i> ⁻¹
StChiAΔCBM	0.73 ^b	0.7 ^b	0.95	2.8 ± 0.1	0.046	0.017	1.0 ± 0.2	0.18	0.18
SgChiA	0.29 ± 0.09	3.1	10.7	4.7 ± 0.4	0.015	0.003	0.39 ± 0.16	0.0355	0.09
PpChitinase	1.1 ± 0.4	10.3	9.6				2.9 ± 0.4	0.007	0.002

^a Monitored by capillary electrophoresis.^b Not tested in this study. Data are from Larsen *et al.* (14).FIGURE 3. Kinetics of hydrolysis by *Salmonella*, *Sodalis*, and *Polysphondylium* chitinases of pNP-(GlcNAc)₂, LacNAc-TMR, and LacdiNAc-TMR at 30 °C.

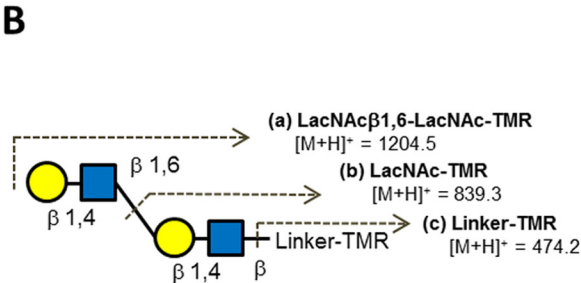
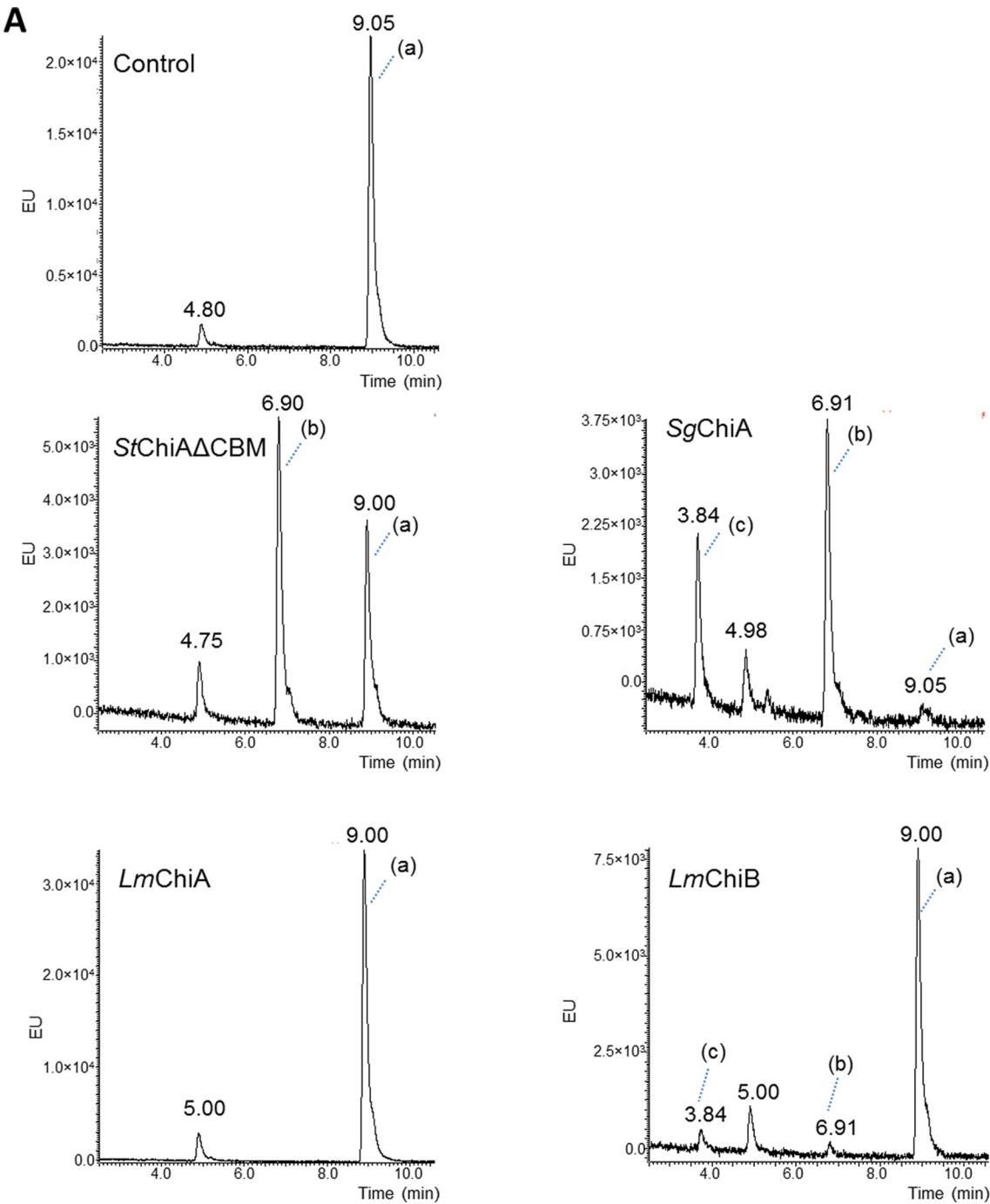
tored by ¹H NMR. LacdiNAc consists of a GalNAc and a GlcNAc unit, and these two units gave distinct anomeric signals in ¹H NMR as can be seen in Fig. 5, which shows the hydrolysis of LacdiNAc-TMR by SgChiA. From the bottom, the first spectrum (Fig. 5A) shows the substrate before adding enzyme with the peak at 4.18 ppm from H1 of GlcNAc and the peak at 4.33 ppm from H1 of GalNAc. The spectra B, C, and D show the formation of the product, LacdiNAc, at 1, 3.5, and 9 h, respectively, after the addition of the enzyme. The peak at 4.37 ppm is the signal from H1 of β-GalNAc, when the reducing end is β-GlcNAc, and the peak at 4.38 ppm is the signal from H1 of β-GalNAc when the reducing end is α-GlcNAc. The signal at 4.55 ppm is the H1 of β-GlcNAc reducing end, and the signal at 5.19 ppm is the H1 of α-GlcNAc reducing end. Note that the α-anomer is formed later than the reducing end β-anomer thus confirming that the reaction occurs with retention of configu-

ration as the reducing end β-anomer is the only product of enzyme-catalyzed steps.

pNP-chitobiose, pNP-(GlcNAc)₂, and chitotriose (GlcNAc)₃ were also shown to be substrates of SgChiA as monitored by NMR with release of β-disaccharide (GlcNAc)₂ from pNP-(GlcNAc)₂ and release of β-monosaccharide and β-disaccharide from the trisaccharide (GlcNAc)₃, and slow mutarotation to α-mono- and α-disaccharide (data not shown). These are analogous to the results previously reported for *Salmonella* StChiAΔCBM (14).

DISCUSSION

We have investigated the affinity of a *Salmonella* chitinase toward a large range of substrates by use of a glycan microarray technique followed by investigations of conversion rates of



C

Enzyme	Composition of TMR Compounds (%)			Hydrolytic Conversion (%)
	(a)	(b)	(c)	
StChiAΔCBM	38	62	0	62
SgChiA	2	65	33	98
LmChiA	100	0	0	n.d.
LmChiB	94	3	3	6

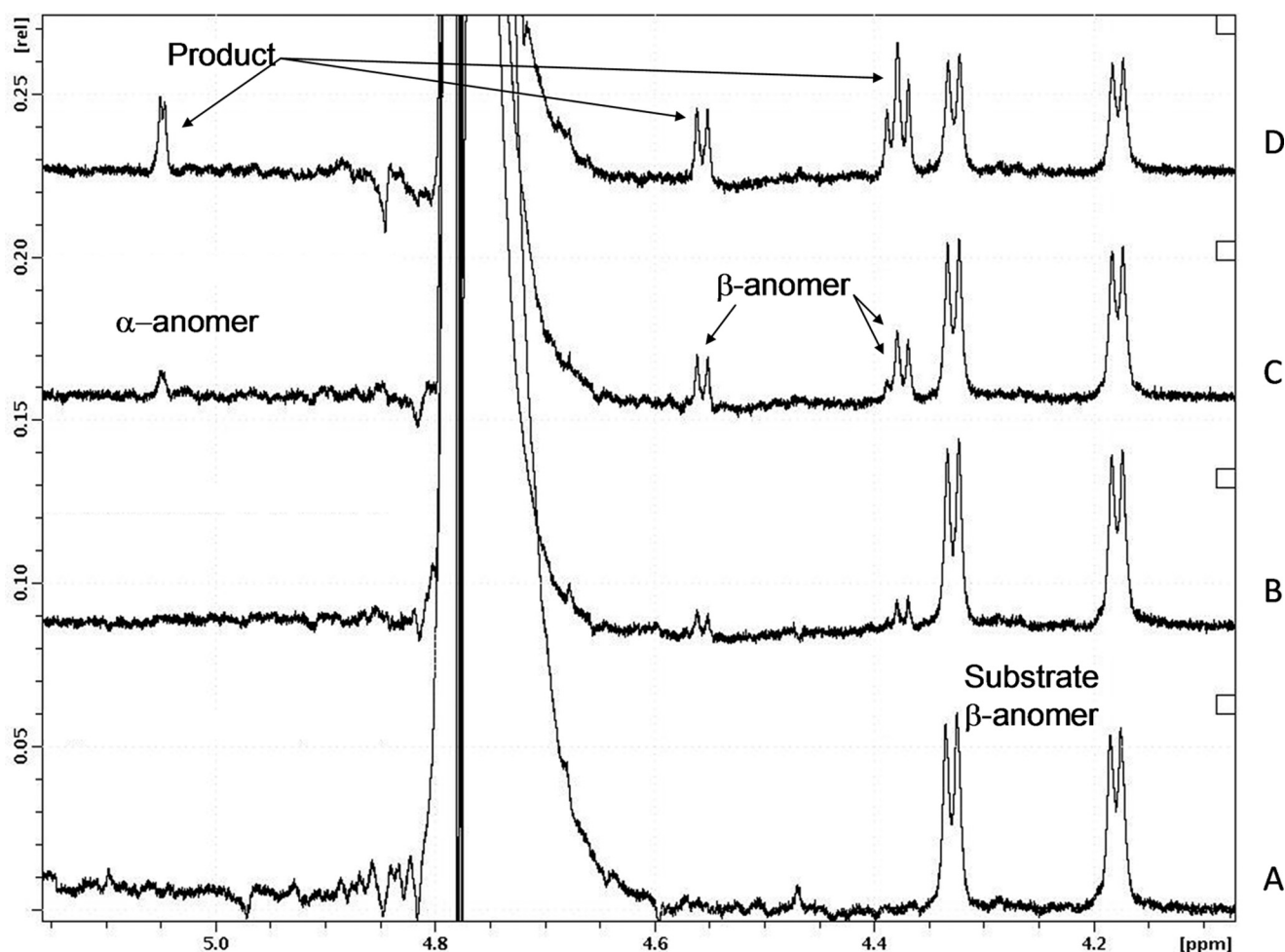


FIGURE 5. Hydrolysis of LacdiNac-TMR by *Sodalis* chitinase (*SgChiA*) monitored by one-dimensional proton NMR showing only the anomeric region. A, substrate before the addition of enzyme. The signal at 4.18 ppm is H1 of GlcNAc and 4.33 ppm H1 of GalNAc. B–D show LacdiNac formed at 1 h (B), 3.5 h (C), and 9 h (D) after the addition of enzyme. The signal at 4.37 ppm is H1 of β -GalNAc, when the reducing end is β -GlcNAc. The signal at 4.38 ppm is H1 of β -GalNAc when the reducing end is α . The signal at 4.55 ppm is β -GlcNAc reducing end, and 5.19 ppm is α -GlcNAc reducing end.

selected substrates by several chitinases and, finally, by screening a small selection of potential biological substrates.

The glycan array scanning highlighted a specific affinity of the full-length *Salmonella* chitinase, *StChiA*, toward various GlcNAc-containing glycans. As expected, chitin-like substrates such as chitopentaose and chitohexaose were targeted by this chitinase. Some GlcNAc-containing glycans of mammalian-like LacNAc structures were also targets (Table 2). LacNAc structures have been identified as terminating glycans of glycoproteins and glycolipids on vertebrate cells and in the human glycome (30, 31). The inclusion of the inactive *Salmonella* variants *StChiA* E223Q and *StChiA* E223Q Δ CBM in the study ensured that a reliable overview of binding affinities was obtained.

Detailed analyses of hydrolysis of the most promising of these mammalian and/or insect-like substrates were examined by use of a wider range of bacterial and eukaryotic chitinases. The chitinases were selected from a broad phylogenetic range and

included the related (Fig. 2) enzymes from the Enterobacteriaceae *Salmonella* Typhimurium and *S. glossinidius* as well as the slime mold *P. pallidum* and the unrelated chitinases from the Firmicute *L. monocytogenes*. Some of the enzymes (the *Listeria* *LmChiB* and *Sodalis* *SgChiA*) encode additional domains annotated as chitin binding domains, whereas others contain only the catalytic domain (*LmChiA*, *StChiA* Δ CBM, *Polysphondylium* *PpChitinase*; Fig. 2). Additional studies are needed to clearly distinguish the respective roles of the family 18 glycosyl hydrolase domain and additional domains. The observation of marginal differences in hydrolytic activities for *LmChiB* and *LmChiB* Δ CBM without the chitin binding domain (Tables 3 and 4) suggest that there is a minimal effect of the chitin binding domain on the hydrolytic activities under the conditions tested.

The enzymatic analyses were performed using the fluorescently labeled model substrates LacdiNac-TMR and LacNAc-TMR. A general activity toward LacdiNac-TMR was observed

FIGURE 4. Ultra-HPLC-mass spectrometry analyses of LacNAc β 1,6-LacNAc-TMR before and after reaction with four different chitinases. A, chromatograms of the reaction mixtures were monitored with a fluorescence detector (λ_{ex} = 540 nm; λ_{em} = 580 nm), and each peak was identified by mass spectrometry. EU, emission units. B, structures of the substrate (a) and the products (LacNAc-TMR (b) and Linker-TMR (c)), corresponding to labels on the chromatograms. The yellow circle and the blue square indicate Gal and GlcNAc, respectively. C, hydrolytic reaction yields based on peak areas of the TMR products formed. For a presentation of the exact structures, please see Fig. 1 and supplemental Fig. 1.

for all of the chitinases examined, except *S. griseus* chitinase, with the release of LacdiNAc from LacdiNAc-TMR. This is consistent with LacdiNAc binding to the -2 and -1 subsites and the hydrophobic linker with TMR in the enzymes $+1$ subsites. The hydrolytic activity toward LacNAc-TMR was much lower, and only the SgChiA and StChiAΔCBM released LacNAc from LacNAc-TMR to any extent within the 2-h incubation time (Table 4). The increased activity toward LacdiNAc-TMR is attributed to the presence of two *N*-acetyl groups, on the reducing end for substrate-assisted catalysis and on the non-reducing end for additional recognition by the respective chitinases. It is notable that the *T. viride* enzyme mixture also exhibited a high degree of hydrolytic activity toward LacdiNAc-TMR. Further analyses are needed to elucidate which *T. viride* chitinase(s) is responsible for this activity and to evaluate the potential biological role. The general activity of chitinases toward a LacdiNAc-terminating substrate is a novel observation only reported previously for the human chitotriosidase (17).

Hydrolysis of LacNAc model substrates is also not commonly reported. In addition to the previous studies of a *Salmonella* chitinase and the human chitotriosidase by the authors of the present paper (14, 17) only two studies have demonstrated such activity expressed by the chitinases in the bacterial species *Amycolatopsis orientalis* (32) and *Bacillus circulans* (33). The *Amycolatopsis* chitinase not only hydrolyzed chitobiose from *p*NP-(GlcNAc)₂ but also released free oligosaccharides and *p*-nitrophenol from Galβ1,4GlcNAcβ1,3Galβ1,4GlcNAcβ-*p*NP, GlcNAcβ1,3Galβ1,4GlcNAcβ-*p*NP, and LacNAc-*p*NP (Galβ1,4GlcNAcβ-*p*NP). LacNAc and LacNAc-*p*NP were not detected in the course of tetrasaccharide hydrolysis, consistent with *p*NP occupying the $+1$ subsite and the tetrasaccharide occupying the enzymes -4 to -1 subsites.

To demonstrate that the activity toward LacdiNAc-TMR and LacNAc-TMR was not a minor side activity of the chitinases, we examined the substrate specificities and Michaelis-Menten kinetic parameters for LacdiNAc-TMR, LacNAc-TMR, and *p*NP-(GlcNAc)₂ for StChiAΔCBM, SgChiA, and PpChitinase.

StChiAΔCBM and SgChiA showed similar trends in hydrolytic activity toward LacNAc-TMR and LacdiNAc-TMR, with an increased activity for LacdiNAc-TMR for both enzymes as seen by the 2–4 times higher k_{cat} values (Table 5). When comparing k_{cat} and k_{cat}/K_m values for LacNAc- and LacdiNAc-TMR to the higher k_{cat} and k_{cat}/K_m values for the chitin pseudo-substrate *p*NP-(GlcNAc)₂, the k_{cat} value for the StChiAΔCBM enzyme with LacdiNAc-TMR was still 25% that of the value observed with *p*NP-(GlcNAc)₂ (Table 5). Even higher k_{cat} values toward LacdiNAc-TMR relative to *p*NP-(GlcNAc)₂ have been observed for the human chitotriosidase (17). The rather low level of activity indicated by the low k_{cat} values observed for the PpChitinase toward LacdiNAc-TMR (Table 5) should be interpreted with caution.

For SgChiA and StChiAΔCBM the LacdiNAc motif may be a potential biological target as this substrate is commonly found in the insect glycome (34). *Salmonella* has been associated with cockroaches, *Chironomus* midges and flies (35–38), and *S. glossinidius* is an endosymbiont of the tsetse fly (*Glossina* spp., Ref. 39).

The presence of chitinases in *P. pallidum* may provide an activity of general importance, such as degradation of yeast prey, as they are also found in another major cellular slime mold genus, *Dictyostelium* (Fig. 2); these two genera mainly belong to different groups within the Dictyostelia (40, 41).

To investigate whether the observed activities toward LacNAc-TMR and LacdiNAc-TMR are biologically relevant, we screened the *Listeria*, *Salmonella*, and *Sodalis* chitinases (*Lm*ChiA and *Lm*ChiB, StChiAΔCBM, and SgChiA, respectively) for activities toward extended substrates including LacNAcβ1–6LacNAcβ-TMR, LacNAcβ1–6(Galβ1–3)GalNAcα-TMR (extended core 2), and LacNAcβ1–2-Man-TMR (Fig. 1). Only LacNAcβ1–6LacNAcβ-TMR was a substrate. It is of interest to note that the two enzymes (StChiAΔCBM and SgChiA) that showed a high catalytic activity toward this substrate both were associated with the phylogenetic group colored in blue and belonging to cluster A (Fig. 2; Ref. 28). Further studies are, however, necessary to demonstrate that this group of chitinases indeed has an increased preference for this target.

The stepwise hydrolysis pattern for LacNAcβ1–6LacNAcβ-TMR is consistent with the terminal LacNAc occupying the -2 and -1 subsites and the inner LacNAc-TMR occupying the $+1$ and $+2$ subsites. The hydrolysis of LacNAcβ1–6LacNAcβ-TMR can be attributed to the flexibility of the β1,6 linkage. The product of hydrolysis is LacNAcβ-TMR, which in turn was further hydrolyzed by SgChiA and *Lm*ChiB enzymes. In this case the linker occupies the $+1$ subsites. The product distribution profiles indicate that the tetrasaccharide is hydrolyzed twice as rapidly as LacNAc-TMR by the SgChiA, whereas they are equally good substrates for *Lm*ChiB. The inability of StChiAΔCBM to further hydrolyze LacNAcβ-TMR might be due to the shorter linker to TMR in this compound ((CH₂)₂) compared with the linker to LacNAc-TMR used for kinetics ((CH₂)₈) or to a greater affinity for tetrasaccharide such that it precludes binding and hydrolysis of LacNAcβ-TMR. This highlights caution in the interpretation of kinetic results when non-natural leaving groups such as fluorescent linkers or the widely used *p*NP are used. The hydrolysis patterns were distinct from those seen for *Amycolatopsis* chitinase, which bound all oligosaccharide substrates with *p*NP in the $+1$ subsite. These results are in agreement with what has recently been observed for the human chitotriosidase (17). It can be concluded that terminal LacNAc or LacdiNAc structure-linked β1,4 or β1,6 in oligosaccharides, glycoproteins, or glycolipids are potential substrates for several chitinases. Especially for the *Salmonella* StChiAΔCBM and *Sodalis* SgChiA, this activity was significant, whereas this was not for the case for the *Listeria* chitinases (Fig. 4). Interestingly, it has been proposed that GH₁₈ enzymes from the human gut bacterial symbiont *Bacteroides thetaiotaomicron* may target LacNAc repeats of O-glycan chains (42).

It can be suggested that for *Salmonella* StChiA and *Sodalis* SgChiA chitinases, glycans that contain either a LacNAc or LacdiNAc motif constitute part of targets that contribute to a biological response, most likely in association with infection of mammalian hosts (*Salmonella*) or symbiosis with insects (*Sodalis*). This hypothesis is in agreement with the observation that the gene expression of the *Salmonella* chitinase is markedly up-regulated during infection of macrophage and epithe-

lial cells (15, 16). We are currently conducting studies aimed at identifying the potential biological substrates for a selection of these enzymes including the *Listeria* and *Salmonella* chitinases.

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Substrate Specificities of Phylogenetically Diverse Chitinases

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A Diverse Range of Bacterial and Eukaryotic Chitinases Hydrolyze the LacNAc (Gal β 1-4GlcNAc) and LacdiNAc (GalNAc β 1-4GlcNAc) Motifs Found on Vertebrate and Insect Cells.

Rikki F. Frederiksen^{1,2}, Yayoi Yoshimura^{2,3}, Birgit G. Storgaard^{1,2,3}, Dafni K. Paspaliari¹, Bent O. Petersen^{3,4}, Kowa Chen⁵, Tanja Larsen¹, Jens Ø. Duus^{3,6}, Hanne Ingmer¹, Nicolai V. Bovin⁷, Ulrika Westerlind⁸, Ola Blixt⁹, Monica M. Palcic³, and Jørgen J. Leisner¹

Supplementary Table 1. Layout of glycan structures of glycan array.

# in the print&plate layouts	Nr.	Spacered form of saccharide	Common name	Short name	Molecular weight	Molecular weight of OS with TFA	V, ul of 10 mM solution
	Monosaccharides						
1	001	Fuc α -sp3	L- α -Fuc	aF	221.3		2
2	002	Gal α -sp3	α -Gal	aA	237.3		2
3	003	Gal β -sp3	β -Gal	bA	237.3	351.3	2
4	004	GalNAc α -sp0	TnSer	TnSer	308.3		2
5	005	GalNAc α -sp3	T _n	Tn	278.3	392.3	2
6	006	GalNAc β -sp3	β -GalNAc	bAN	278.3	392.3	2
7	007	Glc α -sp3	α -Glc	aG	237.3	351.3	2
8	009	Glc β -sp3	β -Glc	bG	237.3		2
9	010	GlcNAc β -sp3	β -GlcNAc	GN	278.3	392.3	2
10	011	GlcNAc β -sp2	β -GlcNAc	GN-C2	264.3	378.3	2
11	012	GlcNAc β -sp7	β -GlcNAc	GN-Ph	312.3		2
12	013	GlcNAc β -sp8	β -GlcNAc	GN-PEG	484.6	598.6	2
13	014	GlcN(Gc) β -sp4	β -GlcN(Gc)	bGN(Gc)	293.3		2
14	015	HOCH ₂ (HOCH) ₄ CH ₂ NH ₂	aminoglucitol	glucitol	181.2		2
15	016	Man α -sp3	α -Man	aM	237.3	351.3	2
16	017	Man α -sp4	α -Man	aM-Gly	236.2		2
17	018	Man β -sp4	β -Man	bM	236.2	350.2	2
18	019	ManNAc β -sp4	β -ManNAc	bMN	277.3	391.3	2

19	020	Rha α -sp3	L- α -Rha	aR	221.3	335.3	2
291	021	GalNAc α -sp4	β -GalNAc	bAN-Gly	277.3		2
292	022	GlcNAc β -sp4	β -GlcNAc	GN-Gly	277.3		2
20	037	3-O-Su-Gal β -sp3	3-O-Su- β -Gal	bA3Su	317.3		2
21	040	4-O-Su-GalNAc β -sp4	4-O-Su- β -GalNAc	bAN4Su-Gly	357.3		2
22	043	6-O-Su-GlcNAc β -sp3	6-O-Su- β -GlcNAc	GN6Su	358.4		2
23	044	GlcA α -sp3	α -glucuronic acid	aGU	251.2		2
24	045	GlcA β -sp3	β -glucuronic acid	bGU	251.2		2
25	046	6-H ₂ PO ₃ Glc β -sp4	β -Glc6P	G6P	316.2		2
26	047	6-H ₂ PO ₃ Man α -sp3	α -Man6P	M6P	339.2 (Na ⁺)		2
27	048	Neu5Ac α -sp3	α -Neu5Ac	Sia	366.4		2
28	049	Neu5Ac α -sp9	α -Neu5AcBn	Sia-Bn	471.5		2
29	050	Neu5Ac β -sp3	β -Neu5Ac	bSia	366.4		2
30	051	No formula available					2
31	052	Neu5Gc α -sp3	α -Neu5Gc	aNeu5Gc	382.4		2
32	053	Neu5Gc β -sp3	β -Neu5Gc	bSia5Gc	382.4		2
33	054	9-NAc-Neu5Ac α -sp3	9-NAc- α -Neu5Ac	9NAcSia	407.4		2
290	055	3-O-Su-GlcNAc β -sp3	3-O-Su- β -GlcNAc-	GN3Su	358.4		2
Disaccharides							
34	071	Fuc α 1-2Gal β -sp3	H _{di}	Hdi	383.4	497.4	2
35	072	Fuc α 1-3GlcNAc β -sp3		Fa3GN	424.5	538.5	2
36	073	Fuc α 1-4GlcNAc β -sp3	Le	Le	424.5		2
37	074	No formula available					2
38	075	Gal α 1-2Gal β -sp3		Aa2A	399.4		2
39	076	Gal α 1-3Gal β -sp3	B _{di}	Bdi	399.4	513.4	2
40	077	Gal α 1-3GalNAc β -sp3	T $\alpha\beta$	Tab	440.5		2
41	078	Gal α 1-3GalNAc α -sp3	T $\alpha\alpha$	Taa	440.5	554.5	2

42	079	No formula available					2
43	080	Gal α 1-3GlcNAc β -sp3		Aa3GN	440.5		2
44	081	Gal α 1-4GlcNAc β -sp3	α -LacNAc	aLN	440.5	554.5	2
45	082	Gal α 1-4GlcNAc β -sp8	α -LacNAc	aLN-PEG	646.7	760.7	2
46	083	Gal α 1-6Glc β -sp4	melibiose	Aa6G	398.4	512.4	2
47	084	Gal β 1-2Gal β -sp3		Ab2A	399.4	513.4	2
48	086	Gal β 1-3GlcNAc β -sp2	Le ^c	LeC-C2	426.4		2
49	087	Gal β 1-3Gal β -sp3		Ab3A	399.4	513.4	2
50	088	Gal β 1-3GalNAc β -sp3	T $_{\beta\beta}$	Tbb	440.5		2
51	089	Gal β 1-3GalNAc α -sp3	TF	TF	440.5	554.5	2
52	090	No formula available					2
53	092	Gal β 1-4Glc β -sp2	Lac	Lac-C2	385.4	499.4	2
54	093	Gal β 1-4Glc β -sp4	Lac	Lac-Gly	398.4		2
55	094	Gal β 1-4Gal β -sp4		Ab4A	398.4	512.4	2
56	095	Gal β 1-4GlcNAc β -OCH ₂ CH ₂ NH(Et)	LacNAc	LN-C2Et	454.5		2
57	097	Gal β 1-4GlcNAc β -sp3	LacNAc	LN	440.5	554.5	2
58	098	Gal β 1-4GlcNAc β -sp5	LacNAc	LN-C8	553.6		2
59	099	Gal β 1-4GlcNAc β -sp8	LacNAc	LN-PEG	646.7	760.7	2
60	100	Gal β 1-6Gal β -sp4		Ab6A	398.4	512.4	2
61	101	GalNAc α 1-3GalNAc β -sp3	Fs-2	Fs2	481.5	595.5	2
62	102	GalNAc α 1-3Gal β -sp3	A _{di}	Adi	440.5	554.5	2
63	103	GalNAc α 1-3GalNAc α -sp3	core 5	core5	481.5		2
64	104	GalNAc β 1-3Gal β -sp3		ANb3A	440.5	554.5	2
65	106	GalNAc β 1-4GlcNAc β -sp3	LacdiNAc	LacdiNAc	481.5		2
66	107	GalNAc β 1-4GlcNAc β -sp2	LacdiNAc	LacdiNAc-C2	467.5	581.5	2
67	109	GalNAc _(furanose) β 1-4GlcNAc β -sp2		ANfb4GN-C2	467.5		2
68	110	Glc α 1-4Glc β -sp3	maltose	Malt2	399.4		2
69	111	Glc β 1-4Glc β -sp4	cellobiose	cello	398.4	512.4	2
70	112	Glc β 1-6Glc β -sp4	gentiobiose	gent	398.4		2

71	113	GlcNAc β 1-3GalNAc α -sp3	core 3	core3	481.5		2
72	114	GlcNAc β 1-3Man β -sp4		GN3M	439.4	553.4	2
73	115	GlcNAc β 1-4GlcNAc β -Asn	chitobiose-Asn	Ch2-Asn	538.5		2
74	117	GlcNAc β 1-4GlcNAc β -sp4	chitobiose	Ch2-Gly	480.5	594.5	2
75	118	GlcNAc β 1-6GalNAc α -sp3	core 6	core6	481.5	595.5	2
76	119	Man α 1-2Man β -sp4		Ma2Mb	398.4	512.4	2
77	120	Man α 1-3Man β -sp4		Ma3M	398.4	512.4	2
78	121	Man α 1-4Man β -sp4		Ma4M	398.4	512.4	2
79	122	Man α 1-6Man β -sp4		Ma6M	398.4		2
80	123	No formula available					2
81	124	Man α 1-2Man α -sp4		Ma2Ma	398.4	512.4	2
293	125	(6-Bn-Gal β 1-4)GlcNAc β -sp2	6'Bn-LacNAc	6'Bn-LN	516.6		2
294	126	6-Bn-Gal α 1-4(6-Bn)GlcNAc β -sp	Bn ₂ - α -LacNAc	Bn2-aLN	606.7		2
295	127	Gal β 1-4Glc β -sp4-Phe	Lac	Lac-Phe	545.6		2
296	128	Gal β 1-4Glc β -sp4-Trp	Lac	Lac-Trp	584.6		2
82	143	Fuc α 1-2(3-O-Su)Gal β -sp3	3-O-Su-H _{di}	Hdi3Su	463.5		2
83	144	Gal β 1-3(6-O-Su)GlcNAc β -sp2	6-O-Su-Le ^c	LeC6Su-C2	506.5		2
84	145	Gal β 1-3(6-O-Su)GlcNAc β -sp3	6-O-Su-Le ^c	LeC6Su	520.5		2
85	146	Gal β 1-4(6-O-Su)Glc β -sp2	6-O-Su-Lac	Lac6Su	465.4		2
86	147	Gal β 1-4(6-O-Su)GlcNAc β -sp3	6-O-Su-LacNAc	LN6Su	520.5		2
87	148	GalNAc β 1-4(6-O-Su)GlcNAc β -sp2	6-O-Su-LacdiNAc	LacdiNAc6Su	547.5		2
88	149	GlcNAc β 1-4(6-O-Su)GlcNAc β -sp2	6-O-Su-chitobiose	Ch2-6Su	547.5		2
89	150	3-O-Su-Gal β 1-3GalNAc α -sp3	3'-O-Su-TF	TF3'Su	520.5		2
90	151	6-O-Su-Gal β 1-3GalNAc α -sp3	6'-O-Su-TF	TF6'Su	520.5		2
91	152	3-O-Su-Gal β 1-4Glc β -sp2	SM3	Lac3'Su	465.3		2
92	153	6-O-Su-Gal β 1-4Glc β -sp2	6'-O-Su-Lac	Lac6'Su	465.3		2
93	154	3-O-Su-Gal β 1-3GlcNAc β -sp2	3'-O-Su-Le ^c	LeC3'Su-C2	506.5		2
94	155	3-O-Su-Gal β 1-3GlcNAc β -sp3	3'-O-Su-Le ^c	LeC3'Su	520.5		2

95	156	3-O-Su-Gal β 1-4GlcNAc β -sp2	3'-O-Su-LacNAc	LN3'Su-C2	506.5		2
96	157	3-O-Su-Gal β 1-4GlcNAc β -sp3	3'-O-Su-LacNAc	LN3'Su	520.5		2
97	158	4-O-Su-Gal β 1-4GlcNAc β -sp2	4'-O-Su-LacNAc	LN4'Su-C2	506.5		2
98	159	4-O-Su-Gal β 1-4GlcNAc β -sp3	4'-O-Su-LacNAc	LN4'Su	520.5		2
99	160	6-O-Su-Gal β 1-3GlcNAc β -sp2	6'-O-Su-Le ^c	LeC6'Su-C2	506.5		2
100	161	6-O-Su-Gal β 1-3GlcNAc β -sp3	6'-O-Su-Le ^c	LeC6'Su	520.5		2
101	162	6-O-Su-Gal β 1-4GlcNAc β -sp2	6'-O-Su-LacNAc	LN6'Su-C2	506.5		2
102	164	GlcA β 1-3GlcNAc β -sp3		GUb3GN	454.3		2
103	165	GlcA β 1-3Gal β -sp3		GUb3A	413.4		2
297	166	GlcA β 1-6Gal β -sp3		GUb6A	413.4		2
104	167	GlcNAc β 1-4-[HOOC(CH ₃)CH]-3-O-GlcNAc β -sp4	GlcNAc-Mur	GN-Mur	552.5		2
105	168	GMDP-Lys	GMDP-Lys	GMDPLys	823.9		2
106	169	Neu5Ac α 2-3Gal β -sp3	GM4	GM4	528.5		2
107	170	Neu5Ac α 2-6Gal β -sp3		Sia6A	528.5		2
108	171	Neu5Ac α 2-3GalNAc α -sp3	3-SiaT _n	3-SiaT _n	569.6		2
109	172	Neu5Ac α 2-6GalNAc α -sp3	SiaT _n	SiaT _n	569.6		2
110	173	Neu5Ac β 2-6GalNAc α -sp3	β -SiaT _n	bSiaT _n	569.6		2
111	174	Neu5Gc α 2-6GalNAc α -sp3	Neu5Gc-T _n	Neu5GcT _n	569.6		2
112	175	Neu5Gc β 2-6GalNAc α -sp3	β Neu5Gc-T _n	bNeu5GcT _n	585.6		2
113	176	3-O-Su-Gal β 1-4(6-O-Su)Glc β -sp2	3',6-di-O-Su-Lac	Lac3',6Su2	567.5 (Na ⁺)		2
114	177	3-O-Su-Gal β 1-4(6-O-Su)GlcNAc β -sp3	3',6-di-O-Su-LacNAc	LN3'6Su2	622.6 (Na ⁺)		2
115	179	6-O-Su-Gal β 1-3(6-O-Su)GlcNAc β -sp2	6,6'-di-O-Su-Le ^c	LeC6,6'Su2	608.5 (Na ⁺)		2
116	180	6-O-Su-Gal β 1-4(6-O-Su)GlcNAc β -sp2	6,6'-di-O-Su-LacNAc	LN66'Su2	608.5 (Na ⁺)		2
117	181	No formula available					2
118	182	3,6-O-Su ₂ -Gal β 1-4GlcNAc β -sp2	3',6'-di-O-Su-LacNAc	LN3'6'Su2	608.5 (Na ⁺)		2
119	183	4,6-O-Su ₂ -Gal β 1-4GlcNAc β -sp2	4',6'-di-O-Su-LacNAc	LN4'6'Su2-C2	608.5 (Na ⁺)		2
120	184	4,6-O-Su ₂ -Gal β 1-4GlcNAc β -sp3	4',6'-di-O-Su-	LN4'6'Su2	622.6 (Na ⁺)		2

			LacNAc				
121	186	Neu5Ac α 2-8Neu5Ac α 2-sp3	(Sia) ₂	(Sia) ₂	679.6 (Na ⁺)		2
122	187	No formula available					2
123	188	No formula available					2
298	189	3,6-O-Su ₂ -Gal β 1-4(6-O-Su)GlcNAc β -sp2	3',6,6'-tri-O-Su-LacNAc	LN3'66'Su3	710.6 (2Na ⁺)		2
299	190	Gal β 1-4(6-P)GlcNAc β -sp2	6P-LacNAc	LN6P	506.4		2
300	191	6-P-Gal β 1-4GlcNAc β -sp2	6'P-LacNAc	LN6'P	506.4		2
301	192	GalNAc β 1-4(6-O-Su)GlcNAc β -sp3	6-O-Su-LacdiNAc	LacdiNAc6Su	561.5		2
302	193	3-O-Su-GalNAc β 1-4GlcNAc β -sp3	3'-O-Su-LacdiNAc	LacdiNAc3'Su	561.5		2
303	194	6-O-Su-GalNAc β 1-4GlcNAc β -sp3	6'-O-Su-LacdiNAc	LacdiNAc6'Su	561.5		2
304	195	6-O-Su-GalNAc β 1-4-(3-O-Ac)GlcNAc β -sp3	6'-Su-3-O-Ac-LacdiNAc	3Ac-LacdiNAc6'Su	603.5		2
305	196	3-O-Su-GalNAc β 1-4(3-O-Su)-GlcNAc β -sp3	3,3'-O-Su ₂ -LacdiNAc	LacdiNAc3,3'Su2	663.5(Na ⁺)		2
306	197	3,6-O-Su ₂ -GalNAc β 1-4-GlcNAc β -sp3	3',6'-Su ₂ -LacdiNAc	LacdiNAc3',6'Su2	663.5(Na ⁺)		2
307	198	4,6-O-Su ₂ -GalNAc β 1-4GlcNAc β -sp3	4',6'-O-Su ₂ -LacdiNAc	LacdiNAc4',6'Su2	663.5(Na ⁺)		2
308	199	4,6-O-Su ₂ -GalNAc β 1-4-(3-O-Ac)GlcNAc β -sp3	4',6'-Su ₂ -3-O-Ac-LacdiNAc	3Ac-LacdiNAc4',6'Su2	705.5(Na ⁺)		2
309	200	4-O-Su-GalNAc β 1-4GlcNAc β -sp3	4'-O-Su-LacdiNAc	LacdiNAc4'Su	561.5		2
310	201	3,4-O-Su ₂ -GalNAc β 1-4-GlcNAc β -sp3	3',4'-Su ₂ -LacdiNAc	LacdiNAc3',4'Su2	663.5(Na ⁺)		2
		Trisaccharides					
124	215	Fuc α 1-2Gal β 1-3GlcNAc β -sp3	Le ^d , H (type 1)	LeD	586.6	700.6	2
125	216	Fuc α 1-2Gal β 1-4GlcNAc β -sp3	H (type 2)	Htype2	586.6	700.6	2
126	217	Fuc α 1-2Gal β 1-3GalNAc α -sp3	H (type 3)	Htype3	586.6	700.6	2
127	219	Fuc α 1-2Gal β 1-4Glc β -sp4	H (type 6)	Htype6	544.5	658.5	2

128	220	Gal α 1-3Gal β 1-4Glc β -sp2		Aa3'Lac-C2	547.5		2
129	221	Gal α 1-3Gal β 1-4Glc β -sp4		Aa3'Lac-Gly	560.5	674.5	2
130	222	Gal α 1-3Gal β 1-4GlcNAc β -sp3	Galili (tri)	Galili3	602.6		2
131	223	Gal α 1-4Gal β 1-4Glc β -sp2	P ^k , Gb3, GbOse ₃	Pk-C2	547.5		2
132	224	Gal α 1-4Gal β 1-4Glc β -sp3	P ^k , Gb3, GbOse ₃	Pk	561.5		2
133	225	Gal α 1-4Gal β 1-4GlcNAc β -sp2	P ₁	P1	588.6		2
134	226	Gal α 1-3 Gal β -sp3 Fuc α 1-2	B _{tri}	Btri	545.5	659.5	2
135	227	Gal α 1-3 Gal β -sp5 Fuc α 1-2	B _{tri}	Btri-C8	658.7	772.7	2
136	228	Gal β 1-2Gal α 1-4GlcNAc β -sp4		Ab2aLN	601.6		2
137	229	Gal β 1-3Gal β 1-4GlcNAc β -sp4		Ab3'LN	601.6		2
138	231	Gal β 1-4GlcNAc β 1-3GalNAc α -sp3		LN3Tn	643.6	757.6	2
139	232	Gal β 1-4GlcNAc β 1-6GalNAc α -sp3		LN6Tn	643.6	757.6	2
140	233	Fuc α 1-4 GlcNAc β -sp3 Gal β 1-3	Le ^a	LeA	586.6	700.6	2
141	234	Gal β 1-4 GlcNAc β -sp3 Fuc α 1-3	Le ^x	LeX	586.6	700.6	2
142	235	GalNAc α 1-3 Gal β -sp3 Fuc α 1-2	A _{tri}	Atri	586.6	700.6	2
143	236	GalNAc α 1-3 Gal β -sp5 Fuc α 1-2	A _{tri}	Atri-C8	699.8		2
144	237	GalNH α 1-3 Gal β -OCH ₂ CH ₂ CH ₂ NHAc Fuc α 1-2	AB _{tri}	ABtri	586.6		2
145	238	GalNAc β 1-4Gal β 1-4Glc β -sp3	GA ₂ , GgOse ₃	GA2	602.6		2
146	239	No formula available					2
147	240	(Glc α 1-4) ₃ β -sp4	maltotriose	(Ga4)3b	560.5	674.5	2
148	241	(Glc α 1-6) ₃ β -sp4	isomaltotriose	(Ga6)3b	560.5	674.5	2

149	242	GlcNAc α 1-3Gal β 1-4GlcNAc β -sp2		GNa3'LN-C2	629.6		2
150	243	GlcNAc α 1-3Gal β 1-4GlcNAc β -sp3		GNa3'LN	643.6		2
151	245	GlcNAc α 1-6Gal β 1-4GlcNAc β -sp2		GNa6'LN	629.6		2
152	246	GlcNAc β 1-2Gal β 1-3GalNAc α -sp3		GN2'TF	643.6	757.6	2
153	247	GlcNAc β 1-3Gal β 1-3GalNAc α -sp3		GN3'TF	643.6		2
154	248	GlcNAc β 1-3Gal β 1-4Glc β -sp2		GN3'Lac	588.6		2
155	249	GlcNAc β 1-3Gal β 1-4GlcNAc β -sp2		GN3'LN-C2	629.6	743.6	2
156	250	GlcNAc β 1-3Gal β 1-4GlcNAc β -sp3		GN3'LN	643.6	757.6	2
157	251	GlcNAc β 1-4Gal β 1-4GlcNAc β -sp2		GN4'LN	629.6		2
158	252	GlcNAc β 1-4GlcNAc β 1-4GlcNAc β -sp4	chitotriose	Ch3	683.6		2
159	253	GlcNAc β 1-6Gal β 1-4GlcNAc β -sp2		GN6'LN	629.6		2
160	254	GlcNAc β 1-6 GalNAc α -sp3 Gal β 1-3	core 2	core2	643.6	757.6	2
161	255	GlcNAc β 1-6 GalNAc α -sp3 GlcNAc β 1-3	core 4	core4	684.7	798.7	2
162	256	No formula available					2
163	258	Man α 1-6 Man β -sp4 Man α 1-3	Man ₃	(Ma)3b	560.5		2
311	259	Gal β 1-4 GlcNAc β -sp3 Gal β 1-3		(Ab)2-3,4GN	602.6		2
164	287	Fuc α 1-4 GlcNAc β -sp3 3-O-Su-Gal β 1-3	Su-Le ^a	3'SuLeA	666.7		2
165	288	3-O-Su-Gal β 1-4 GlcNAc β -sp3 Fuc α 1-3	Su-Le ^x	3'SuLeX	666.7		2
166	289	Neu5Ac α 2-6 GalNAc α -sp3 Gal β 1-3	6-SiaTF	6SiaTF	731.7		2
167	290	Neu5Ac α 2-6 GalNAc α -sp3 Gal α 1-3		A3a(Sia)Tn	731.7		2

168	291	No formula available					2
169	292	Neu5Ac α 2-3Gal β 1-3GalNAc α -sp3	3'-Sia-TF	Sia3'TF	731.7		2
170	293	Neu5Ac α 2-3Gal β 1-4Glc β -sp3	3'SL	3'SL	690.7		2
171	294	Neu5Ac α 2-3Gal β 1-4Glc β -sp4	3'SL	3'SL-Gly	689.6		2
172	295	Neu5Ac α 2-6Gal β 1-4Glc β -sp2	6'SL	6'SL-C2	676.6		2
173	296	Neu5Ac α 2-6Gal β 1-4Glc β -sp4	6'SL	6'SL-Gly	689.6		2
174	297	Neu5Ac β 2-6Gal β 1-4Glc β -sp2	β -6'SL	b6'SL	676.6		2
175	298	Neu5Ac α 2-3Gal β 1-4GlcNAc β -sp3	3'SLN	3'SLN	731.7		2
176	299	Neu5Ac α 2-3Gal β 1-3GlcNAc β -sp3	3'-SiaLe ^c	3'SiaLeC	731.7		2
177	300	Neu5Ac α 2-6Gal β 1-4GlcNAc β -sp3	6'SLN	6'SLN	731.7		2
178	301	Neu5Ac α 2-6Gal β 1-4GlcNAc β -sp8	6'SLN	6'SLN-PEG	938.0		2
179	302	Neu5Ac β 2-6Gal β 1-4GlcNAc β -sp3	β 6'SLN	b6'SLN	731.7		2
180	304	Neu5Gc α 2-6Gal β 1-4GlcNAc β -sp3	6'SLN(Gc)	6'SLN(Gc)	747.7		2
181	305	Neu5Gc β 2-6Gal β 1-4GlcNAc β -sp3	β 6'SLN(Gc)	b6'SLN(Gc)	747.7		2
182	306	9-NAc-Neu5Ac α 2-6Gal β 1-4GlcNAc β -sp3		9NAc-6'SLN	788.8		2
183	307	KDN α 2-3Gal β 1-3GlcNAc-sp2	KDN-Le ^C	KDN-LeC	676.63		2
184	308	KDN α 2-3Gal β 1-4GlcNAc-sp2	KDN-LacNAc	KDN-LN	676.63		2
185	309	Neu5Ac α 2-6 GalNAc α -sp3 Neu5Ac α 2-3		Sia2-3,6Tn	882.8 (Na ⁺)		2
186	310	3'SiaLacNAc β -OCH ₂ CH ₂ CH ₂ NH-(3'SiaLacNAc-amide-sp3)		(3'SLN) ₂	1445.4		2
187	313	4-O-Su-Neu5Ac α 2-3Gal β 1-4GlcNAc β -sp3	4''-Su-3'SLN	3'SLN4''Su	833.8 (Na ⁺)		2
188	314	9-O-Su-Neu5Ac α 2-3Gal β 1-4GlcNAc β -sp3	9''-Su-3'SLN	3'SLN9''Su	833.8 (Na ⁺)		2
189	315	Neu5Ac α 2-3Gal β 1-4-(6-O-Su)GlcNAc β -sp3	6-Su-3'SLN	3'SLN6Su	833.8 (Na ⁺)		2
190	317	Neu5Ac α 2-3Gal β 1-3-(6-O-Su)GalNAc α -sp3	6-Su-3'SiaTF	3'SiaTF6Su	833.8 (Na ⁺)		2
191	318	Neu5Ac α 2-6Gal β 1-4-(6-O-Su)GlcNAc β -sp3	6-Su-6'SLN	6'SLN6Su	833.8 (Na ⁺) _t		2
192	319	Neu5Ac α 2-3-(6-O-Su)Gal β 1-4GlcNAc β -sp3	6'-Su-3'SLN	3'SLN6'Su	833.8 (Na ⁺)		2
193	320	4-O-Su-Neu5Ac α 2-3-(6-O-Su)Gal β 1-4GlcNAc β -sp3	6',4''-Su ₂ -3'SLN	3'SLN6',4''Su ₂	935.8 (2Na ⁺)		2
194	321	(Neu5Ac α 2-8) ₃ -sp3	(Sia) ₃	(Sia) ₃	992.9 (2Na ⁺)		2
195	322	(Neu5Ac α 2-8) ₃ β -sp3	(Sia) ₃ β	(Sia) ₃ b	992.9 (2Na ⁺)		2

	Tetrasaccharides						
196	359	Gal α 1-3 Gal β 1-3GlcNAc β -sp3 Fuc α 1-2	B (type 1)	Btype1	748.7		2
197	360	Gal α 1-3 Gal β 1-4GlcNAc β -sp3 Fuc α 1-2	B (type 2)	Btype2	748.7		2
198	361	Gal α 1-3 Gal β 1-4GlcNAc β -sp2 Fuc α 1-2	B (type 2)	Btype2-C2	734.7		2
199	362	Gal α 1-3 Gal β 1-3GalNAc α -sp3 Fuc α 1-2	B (type 3)	Btype3	748.7		2
200	363	Gal α 1-3 Gal β 1-3GalNAc β -sp3 Fuc α 1-2	B (type 4)	Btype4	748.7	862.7	2
201	364	Gal α 1-3Gal β 1-4 GlcNAc β -sp3 Fuc α 1-3	α GalLe ^x	aGalLeX	748.7		2
202	365	No formula available					2
203	366	GalNAc α 1-3 Gal β 1-3GlcNAc β -sp3 Fuc α 1-2	A (type 1)	Atype1	789.8	903.8	2
204	367	GalNAc α 1-3 Gal β 1-4GlcNAc β -sp2 Fuc α 1-2	A (type 2)	Atype2-C2	775.8	889.8	2
205	368	GalNAc α 1-3 Gal β 1-4GlcNAc β -sp3 Fuc α 1-2	A (type 2)	Atype2	789.8		2
206	369	No formula available					2
207	371	Fuc α 1-4 GlcNAc β -sp3 Fuc α 1-2Gal β 1-3	Le ^b	LeB	732.7	846.7	2
208	372	Fuc α 1-2Gal β 1-4 GlcNAc β -sp3 Fuc α 1-3	Le ^y	LeY	732.7	846.7	2
209	373	Gal α 1-3Gal β 1-4GlcNAc β 1-3Gal β -sp3	Galili (tetra)	Galili4	764.7		2
312	374	Gal α 1-4 Gal β 1-4GlcNAc β -sp3		Aa2-3',4'LN	764.7		2

		Gal α 1-3					
210	375	No formula available					2
211	376	Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc β -sp4	LNT	LNT	763.7	877.7	2
212	377	Gal β 1-3GlcNAc β 1-3Gal β 1-3GlcNAc β -sp2		LeCb3'LeC	791.8	905.8	2
213	378	No formula available					2
214	379	Gal β 1-3GlcNAc β 1-3Gal β 1-4GlcNAc β -sp3		LeCb3'LN	805.8	919.8	2
215	380	No formula available					2
216	381	Gal β 1-3GlcNAc β 1-6Gal β 1-4GlcNAc β -sp2		LeCb6'LN	791.8	905.8	2
217	382	Gal β 1-3GalNAc β 1-4Gal β 1-4Glc β -sp3	Asialo-GM1	aGM1	764.7		2
218	383	Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc β -sp4	LNnT	LNnT	763.7		2
219	384	Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β -sp2	i	LNb3'LN-C2	791.7		2
220	385	Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β -sp3	i	LNb3'LN	805.8		2
221	386	No formula available					2
222	387	Gal β 1-4GlcNAc β 1-6Gal β 1-4GlcNAc β -sp2		LNb6'LN	791.7		2
223	388	Gal β 1-4GlcNAc β 1-6 GalNAc α -sp3 Gal β 1-3		LNb6TF	805.8	919.8	2
224	389	GalNAc β 1-3Gal α 1-4Gal β 1-4Glc β -sp3	Gb4, P	Gb4	764.7		2
225	390	(Glc α 1-4) $_4\beta$ -sp4	maltotetraose	(Ga4)4b	722.7	836.7	2
226	391	(Glc α 1-6) $_4\beta$ -sp4	isomaltotetraose	(Ga6)4b	722.7		2
313	394	GlcNAc β 1-4 Gal β 1-4GlcNAc β -sp2 GlcNAc β 1-3		GN2-3',4'LN	832.8		2
314	395	GlcNAc β 1-6 Gal β 1-4GlcNAc β -sp2 GlcNAc β 1-3	Tk	Tk	832.8		2
227	396	No formula available					2
315	397	Gal β 1-3GlcN(Fm) β 1-3Gal β 1-4GlcNAc β -sp3	formylLe ^C β 3LN	LeC(Fm)b3'LN	791.8		
228	419	3-O-SuGal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β -sp3		(3'SuLN)3'LN	907.8		2
229	420	4-O-SuGal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β -sp3		(4'SuLN)3'LN	907.8		2
230	421	GalNAc β 1-4 Gal β 1-4Glc β -sp2 Neu5Ac α 2-3	GM2	GM2	879.8		2

231	422	Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-3Gal β -sp3		3'SLNb3A	893.9		2
232	423	Neu5Ac α 2-3Gal β 1-4 GlcNAc β -sp3 Fuc α 1-3	SiaLe ^x	SiaLeX	877.9		2
233	425	No formula available, SleXfuc-beta					2
234	426	Fuc α 1-4 GlcNAc β -sp3 Neu5Ac α 2-3Gal β 1-3	SiaLe ^a	SiaLeA	877.9		2
235	427	Fuc α 1-4 GlcNAc β -sp4 Neu5Ac α 2-3Gal β 1-3	SiaLe ^a	SiaLeA-Gly	876.8		2
236	428	Neu5Ac α 2-3Gal β 1-4 6-O-Su-GlcNAc β -sp3 Fuc α 1-3		SiaLeX6Su	979.9 (Na ⁺)		2
237	429	Neu5Ac α 2-3(6-O-Su)Gal β 1-4 GlcNAc β -sp3 Fuc α 1-3		SiaLeX6'Su	979.9 (Na ⁺)		2
238	430	No formula available					2
239	431	No formula available					2
240	432	No formula available					2
241	433	Neu5Ac α 2-6 GalNAc α -sp3 Neu5Ac α 2-3Gal β 1-3	Sia ₂ -TF	Sia2-3',6TF	1044.9 (Na ⁺)		2
242	434	Neu5Ac α 2-8Neu5Ac α 2-3Gal β 1-4Glc β -sp4	GD3	GD3	1002.9 (Na ⁺)		2
243	435	No formula available					2
244	436	No formula available					2
		Penta-nona saccharides					
245	479	Fuc α 1-2Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc β -sp4	LNFP-I	Htype1Lac	909.9	1023.9	2
246	480	Fuc α 1-2Gal β 1-3GlcNAc β 1-3Gal β 1-4GlcNAc β -sp2	H(type 1) penta	Htype1LN	937.91		2
247	481	Gal α 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc β -sp4	Galili (penta)	Galili5	925.8	1039.8	2
248	483	Gal α 1-3 Gal β 1-4 Fuc α 1-2 GlcNAc β -sp3 Fuc α 1-3	BLe ^y	BLeY	894.9		2
249	485	No formula available					2
250	486	No formula available					2

251	488	Galβ1-4GlcNAcβ1-6 GalNAcα-sp3 Galβ1-4GlcNAcβ1-3		LN2-3,6Tn	1008.9		2
252	489	GlcNAcβ1-6 Galβ1-4GlcNAc-sp2 Galβ1-4GlcNAcβ1-3		LN3'(GN6')LN	994.9		2
253	490	Galβ1-4GlcNAcβ1-6 Galβ1-4GlcNAcβ-sp2 GlcNAcβ1-3		LN6'(GN3')LN	994.9		2
254	492	(Glcα1-6) ₅ β-sp4	isomaltopentaose	(Ga6)5b	884.8	998.8	2
255	493	(GlcNAcβ1-4) ₅ β-sp4	chitopentaose	Ch5	1090.0		2
256	495	Manα1-6 Manα1-6 Manα1-3 Manβ-sp4 Manα1-3	Man5	(Ma)5b	966.9		2
257	496	Fucα1-4 GlcNAcβ1-3Galβ1-4Glcβ-sp4 Fucα1-2Galβ1-3	Le ^b -Lac	LeBLac	1056.0	1170.0	2
316	497	Fucα1-2Galβ1-4 GlcNAcβ1-3Galβ1-4Glcβ-sp4 Fucα1-3	Le ^Y -Lac	LeYLac	1056.0		2
258	498	(Galβ1-4GlcNAcβ1-3) ₃ -sp3	(LN) ₃	(LNb3') ₃	1171.1	1285.1	2
259	499	Galβ1-4GlcNAcβ1-6 Galβ1-4GlcNAc-sp2 Galβ1-4GlcNAcβ1-3	I	LN2-3',6'LN	1157.1		2
260	502	(Glcα1-6) ₆ β-sp4	maltohexaose	(Ga6)6b	1046.9	1160.9	2
261	503	(GlcNAcβ1-4) ₆ -sp4	chitohexaose	Ch6	1293.3		2
262	504	(A-GN-M) ₂ -3,6-M-GN-GNβ-sp4	9-OS	9-OS	1697.6		2
263	505	(GN-M) ₂ -3,6-M-GN-GNβ-sp4	7-OS	7-OS	1373.3		2
264	527	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-sp2	3'SLN-LacNAc	3'SLN-LN	1083.02		2
265	528	Neu5Acα2-3Galβ1-4 GlcNAcβ1-3Galβ-sp3 Fucα1-3	SiaLe ^x -3Gal	SiaLeX3A	1040.0		2
266	529	Neu5Acα2-6 GlcNAcβ1-3Galβ1-4Glcβ-sp4 Galβ1-3	LSTb	LSTb	1055.0		2
267	530	No formula available (Neu5Acα2-3Galβ1) ₂ -3,4-GlcNAc-sp3					2

268	531	GalNAcβ1-4 Galβ1-4Glc-sp2 Neu5Acα2-8Neu5Acα2-3	GD2	GD2	1193.1 (Na ⁺)		2
269	532	Neu5Acα2-8Neu5Acα2-8Neu5Acα2-3Galβ1-4Glc-sp2	GT3	GT3	1303.1 (2Na ⁺)		2
270	533	GalNAcβ1-4 Galβ1-4Glc-sp2 (Neu5Acα2-8) ₂ Neu5Acα2-3	GT2	GT2	1506.3 (2Na ⁺)		2
	Higher oligosaccharides						
271	624	(GlcAβ1-4GlcNAcβ1-3) ₈ -NH ₂ -ol	hyaluronic acid	HyalU-ol	3207.5 (7Na ⁺)		2
272	626	(Sia2-6A-GN-M) ₂ -3,6-M-GN-GN-NH ₂ -ol	11-OS (aminoalditol)	11-OS-ol	2247.1 (Na ⁺)		2
273	627	(Sia2-6A-GN-M) ₂ -3,6-M-GN-GNβ-sp4	11-OS, YDS	11-OS	2302.1 (Na ⁺)		2
317	900	H-(Gly) ₆ -NH ₂ Gly6-amide, linear	Gly6	Gly6	359.3		2
274	comp 1	GlcNAcβ2Manα6 GlcNAcβ4Manβ4GlcNAcβ4GlcNAcβ-sp6 GlcNAcβ2Manα3		NGA2B	1592.62		53.37 nmol
275	comp 2	Galβ4GlcNAcβ2Manα6 GlcNAcβ4Manβ4GlcNAcβ4GlcNAcβ-sp6 Galβ4GlcNAcβ2Manα3		4'-NA2B	1916.79		44.34 nmol
276	comp 3	Neu5Acα3Galβ4GlcNAcβ2Manα6 GlcNAcβ4Manβ4GlcNAcβ4GlcNAcβ-sp6 Neu5Acα3Galβ4GlcNAcβ2Manα3		3'-A2B	2499.30		34.01
280	comp 4	Neu5Acα6Galβ4GlcNAcβ2Manα6 GlcNAcβ4Manβ4GlcNAcβ4GlcNAcβ-sp6 Neu5Acα6Galβ4GlcNAcβ2Manα3		6'-A2B	2499.30		34.01
281	comp 5	GlcNAcβ2Manα6 GlcNAcβ4Manβ4GlcNAcβ4GlcNAcβ-sp6 GlcNAcβ4(GlcNAcβ2)Manα3		NGA3B(2-4)	1795.70		49.03
282	comp 6	Galβ4GlcNAcβ2Manα6 GlcNAcβ4Manβ4GlcNAcβ4GlcNAcβ-sp6 Galβ4GlcNAcβ4(Galβ4GlcNAcβ2)Manα3		4'-NA3B(2-4)	2282.12		38.56
277	comp 7	Neu5Acα3Galβ4GlcNAcβ2Manα6 GlcNAcβ4Manβ4GlcNAcβ4GlcNAcβ-sp6 Neu5Acα3Galβ4GlcNAcβ4(Neu5Acα3Galβ4GlcNAcβ2)Manα3		3'-A3B(2-4)	3155.89		27.88
278	comp 8	Neu5Acα6Galβ4GlcNAcβ2Manα6 GlcNAcβ4Manβ4GlcNAcβ4GlcNAcβ-sp6 Neu5Acα6Galβ4GlcNAcβ4(Neu5Acα6Galβ4GlcNAcβ2)Manα3		6'-A3B(2-4)	3155.89		27.88
279	comp 9	GlcNAcβ6(GlcNAcβ2)Manα6 GlcNAcβ4Manβ4GlcNAcβ4GlcNAcβ-sp6 GlcNAcβ2Manα3		NGA3B(2-6)	1795.70		46.22
286	comp 10	Galβ4GlcNAcβ6(Galβ4GlcNAcβ2)Manα6 GlcNAcβ4Manβ4GlcNAcβ4GlcNAcβ-sp6 Galβ4GlcNAcβ2Manα3		4'-NA3B(2-6)	2282.12		36.37
287	comp 11	Neu5Acα3Galβ4GlcNAcβ6(Neu5Acα3Galβ4GlcNAcβ2)Manα6 GlcNAcβ4Manβ4GlcNAcβ4GlcNAcβ-sp6 Neu5Acα3Galβ4GlcNAcβ2Manα3		3'-A3B(2-6)	3155.89		26.30
288	comp	Neu5Acα6Galβ4GlcNAcβ6(Neu5Acα6Galβ4GlcNAcβ2)Manα6 GlcNAcβ4Manβ4GlcNAcβ4GlcNAcβ-sp6		6'-A3B(2-6)	3155.89		26.30

	12	Neu5Ac α 6Gal β 4GlcNAc β 2Man α 3					
283	comp 13	GlcNAc β 6(GlcNAc β 2)Man α 6 GlcNAc β 4Man β 4GlcNAc β 4GlcNAc β -sp6 GlcNAc β 4(GlcNAc β 2)Man α 3		NGA4B	1998.89		40.02
284	comp 14	Gal β 4GlcNAc β 6(Gal β 4GlcNAc β 2)Man α 6 GlcNAc β 4Man β 4GlcNAc β 4GlcNAc β -sp6 Gal β 4GlcNAc β 4(Gal β 4GlcNAc β 2)Man α 3		4'-NA4B	2647.46		30.21
285	comp 15	Neu5Ac α 3Gal β 4GlcNAc β 6(Neu5Ac α 3Gal β 4GlcNAc β 2)Man α 6 GlcNAc β 4Man β 4GlcNAc β 4GlcNAc β -sp6 Neu5Ac α 3Gal β 4GlcNAc β 4(Neu5Ac α 3Gal β 4GlcNAc β 2)Man α 3		3'-A4B	3812.47		20.98
289	comp 16	Neu5Ac α 6Gal β 4GlcNAc β 6(Neu5Ac α 6Gal β 4GlcNAc β 2)Man α 6 GlcNAc β 4Man β 4GlcNAc β 4GlcNAc β -sp6 Neu5Ac α 6Gal β 4GlcNAc β 4(Neu5Ac α 6Gal β 4GlcNAc β 2)Man α 3		6'-A4B	3812.47		20.98

Each vial contains 2 μ l 10 mM oligosaccharide solution (20 nmol of the OS) in 20% (v/v) DMSO in H₂O for 200 μ l of the 100 μ M solution preparation

Comp # - compounds from Carlo Unvergatz (obtained in October 2009-11-28)

Concentration of the controls

Short name	Controls	Initial concentration	1st conc. or dilution	2nd conc. or dilution
StrCy3	Streptavidin-Cy3 (Invitrogen)	1 mg/ml	50 ug/ml	10 ug/ml
hIgG	human IgG (Sigma)	10mg/ml	500 ug/ml	100 ug/ml
mIgG	murine IgG (Sigma)	1 mg/ml	500 ug/ml	100 ug/ml
FGF23	20-mer peptide-biotin (FGF23) (Blixt)	10 mg/ml	100 ug/ml	20 ug/ml
iM-FITC	2-mer peptide-FITC (iM) (Blixt)	?	1 : 2	1 : 10

LEGEND:

sp2 = C2 = -O(CH₂)₂NH₂

sp3 = C3 = -O(CH₂)₃NH₂

sp4 =Gly = -NHCOCH₂NH₂

sp5 =C8 = -O(CH₂)₃NH-CO(CH₂)₅NH₂

sp6 = Ox = -N(Me)O(CH₂)₃NH₂

sp7 = Ph = -OC₆H₄-p-NH₂

sp8 = PEG = -(OCH₂CH₂)₆NH₂

sp9 = Bn = -OCH₂C₆H₄-p-NHCOCH₂NH₂

sp0 = other spacers: Asn, Ser, C2Et (see details in the column "Spacered form of saccharide")

Asn = asparagine, $\text{NH-CO-CH}_2\text{CH}(\text{COOH})\text{NH}_2$

Ser = serine, $-\text{OCH}_2\text{CH}(\text{COOH})\text{NH}_2$

C2Et = $-\text{O}(\text{CH}_2)_2\text{NHEt}$

A = Gal

AN = GalNAc

Ch = chito

F = L-Fuc

G = Glc

Gc = glycolyl

GN = GlcNAc β

R = Rha

i = iso

Lac = lactose

LN = N-acetyllactosamine

M = Man

MN = ManNAc

Malt = maltose

OS = oligosaccharide

P = phosphate

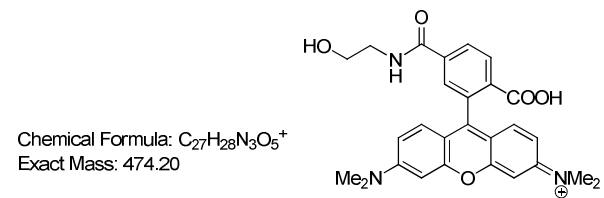
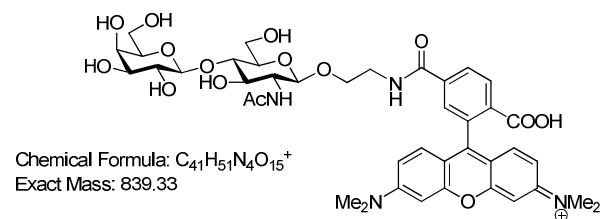
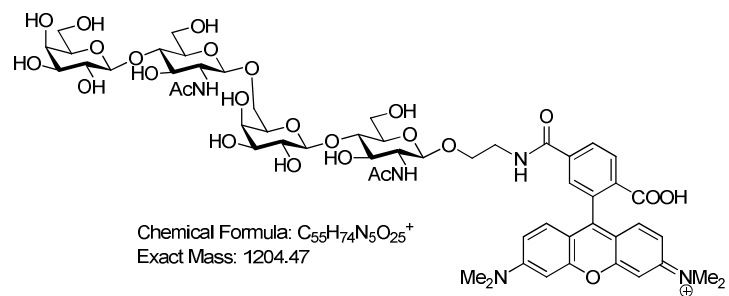
S = Sia = Neu5Ac α

Su = sulfate

Tn = GalNAc α

U = uronic acid

Supplementary Fig 1. Structures and molecular weights of LacNAc β 1,6-LacNAc-TMR, and products of chitinase hydrolysis.



A Diverse Range of Bacterial and Eukaryotic Chitinases Hydrolyzes the LacNAc (Gal β 1–4GlcNAc) and LacdiNAc (GalNAc β 1–4GlcNAc) Motifs Found on Vertebrate and Insect Cells

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